A COMPARISON OF THE BEHAVIORAL AND PHYSIOLOGICAL ASPECTS OF HYDROMINERAL BALANCE IN FISCHER 344 AND SPRAGUE-DAWLEY RATS

By

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Sprague-Dawley (SD), as well as many other strains of rats, show a spontaneous preference and appetite for sodium chloride (NaCl) solutions. This may be related to the renin-angiotensin-aldosterone system (RAAS). Recently a strain of rats (Fischer 344; F344) has been described which have no preference for NaCl. This strain is also resistant to salt-related hypertension.

The present experiments attempt to understand these strain differences by comparing a number of behavioral and physiological parameters of the RAAS. The results of the behavioral studies showed the F344 rats consumed significantly less water and NaCl when compared to SD rats, after administration of drugs believed to work via activation of the RAAS (such as isoproterenol and serotonin) as well as after direct administration of various components of the RAAS (such as angiotensin I and angiotensin III).

However, the F344 rats imbibed water and NaCl solutions similar to the intakes of SD rats after administration of angiotensin II.

Physiological examination of the RAAS in F344 and SD rats revealed no significant differences in plasma renin activity (PRA) nor plasma renin concentration (PRC) after stimulation with isoproterenol. Additionally, measurement of B-adrenergic receptor binding sites in the heart, kidney and cerebral cortex revealed no significant differences between the two strains of rats. Measurement of angiotensin converting enzyme (ACE) activity in several tissues from both strains showed some strain differences but these did not appear to be related to the described behavioral differences.

Measurement of blood pressure and heart rate in F344 and SD rats after administration of isoproterenol showed similar increases in heart rate between the two strains, but a 2-3 times greater decrease in blood pressure in the SD rats.

It is hypothesized that the greater decrease in blood pressure in SD rats may underlie their greater drinking response to the same dose of isoproterenol. The present results are in agreement with the hypothesis that activation of the RAAS leads to a maximal dipsogenic response only when blood pressure is below normal.

CHAPTER 1

INTRODUCTION

Physiology of Fluid Homeostasis

All of the cells in the body depend on water to function properly. Fluids are lost from the body via common processes such as breathing, salivating, sweating, urinating and defecating. There are two major mechanisms by which hydromineral and cardiovascular homeostasis can be maintained: (a) behaviorally by consumption of either water or salt solutions, and (b) physiologically by activation of the renin-angiotensin-aldosterone system (RAAS).

Drinking has often been divided into two main types. Primary or "homeostatic" drinking is said to occur in response to body fluid imbalances between the intracellular and extracellular fluid compartments. An example of primary drinking is the drinking which follows excessive sweating when there has been a loss of fluid from both the extracellular and intracellular fluid compartments. Secondary or "nonhomeostatic" drinking is said to occur when there is no identifiable change in body fluid compartments and it does not reduce any physiological deficit (Fitzsimons, 1979; Rolls and Rolls, 1982; Moore-Ede, 1986). An example of secondary drinking is schedule-induced polydipsia (SIP). In SIP, rats are reduced in body weight (e.g., to 80%) with water available ad libitum, and are given daily feeding sessions in which food is present on a variable interval schedule (typically 1 minute). The results of this type of contingency schedule is that the rats drink excessive amounts of water between food deliveries (Falk, 1961).

In all living systems, water is divided into two main compartments: the intracellular and extracellular fluid compartments. All the water inside the cells is the intracellular fluid while everything outside the cells is extracellular fluid. The extracellular fluid is further divided into the interstitial fluid (between the cells), the vasculature (blood plasma) and two slowly equilibrating compartments which consist of transcellular elements, cartilage and bone. During most instances of extracellular fluid exchange, it is the interstitial and vasculature components which are responding. The transcellular water (water in closed cavities such as joints or cerebral ventricles) and water found in the matrices of bone or cartilage are relatively inaccessible during fluid shifts. The intracellular fluid comprises approximately 33% of the body weight, the interstitial fluid is approximately 12% body weight, the blood plasma is approximately 4.5% body weight and the transcellular elements, cartilage and bone approximately 10.5% (Duling, 1983a). Although the blood plasma accounts for only 4.5% of the body weight, excessive loss of blood or plasma water can lead to circulatory collapse and eventual death.

Intracellular and extracellular fluids are also distinguishable by the composition of the fluids in each compartment. Extracellular fluid has more sodium (Na^+) and chloride (Cl^-) ions while intracellular fluid has more potassium (K^+) ions and protein anions. The separation of ions between intracellular and extracellular fluid compartments is due to the selective permeability of the cell membrane, capillary wall, and the sodium-potassium pump (see Duling, 1983a; Carlson, 1986; and Shepherd, 1983 for extended discussion).

The anatomical and physiological differences between the intracellular and extracellular fluid compartments are also of significance because they allow for selective manipulation of either the intracellular or extracellular fluid compartment. For example, if the Na^+ concentration of the extracellular fluid is increased by administering hypertonic sodium chloride (NaCl), there will be a withdrawal of water from inside the cells (cellular dehydration) which, in turn, can stimulate drinking. Depletion of extracellular fluid (extracellular dehydration) can be accomplished by hemorrhage or injection of colloid materials such as polyethylene glycol (PEG) which sequesters fluid at the site of the injection. Intravenous (iv) injections of isotonic NaCl can selectively increase the volume of the extracellular fluid compartment. Water deprivation can decrease both the intracellular and extracellular fluid compartments while ingestion of water expands both the intracellular and extracellular fluid compartments. Ultimately, all of these processes, working either singly or in combination, will result in water or salt consumption.

Neural Mechanisms for Fluid Homeostasis

Intracellular Mechanisms

The lateral preoptic area (LPO) may be involved in the detection of cellular dehydration. Peck and Novin (1971) and Blass and Epstein (1971) independently concluded that the LPO was the site for osmoreceptors for thirst.

Peck and Novin (1971), using rabbits, showed that lesions of the LPO resulted in deficits in drinking to cellular dehydration induced by iv injections of hypertonic NaCl, but the same animals would drink normally to water deprivation. In addition, microliter injections within the LPO yielded drinking to NaCl and sucrose administration but not urea.

Blass and Epstein (1971), showed that rats with bilateral LPO lesions had deficits in drinking to cellular dehydration induced by intraperitoneal (ip) injections of hypertonic NaCl but the same rats drank normally to hypovolemia produced by administration of PEG. In addition, they found injections of hyperosmotic NaCl and sucrose into the LPO elicited drinking in satiated rats, but urea was not effective. Furthermore, they showed that thirst induced by cellular dehydration could be temporarily suppressed by microliter injections of water into the LPO. Therefore, both Peck and Novin and Blass and Epstein concluded that LPO lesions produce selective deficits in cellular dehydration drinking.

Electrophysiological studies have revealed that cells in the LPO are activated by intracarotid infusions of hyperosmotic solutions (Malmo and Malmo, 1979; Malmo and Mundl, 1975). Therefore, this convergence of information suggests that the LPO contains osmoreceptors for the detection of cellular dehydration.

Another area believed to be involved in detection of cellular dehydration is the nucleus circularis, in the hypothalamus near the walls of the third ventricle. Hatton (1976) has found that the nucleus circularis sends axons to the supraoptic nucleus (which controls secretion of ADH). Hatton reported that electrical stimulation of the nucleus circularis, but not adjacent tissue, caused water to be retained. Hatton also found that water deprivation led to an increase in the number of nucleoli in the cells of the nucleus circularis (suggesting increased protein synthesis). In addition, he found that destruction of the nucleus circularis produces deficits in release of ADH in response to increased osmotic pressure. Therefore, the nucleus circularis is thought to contain osmoreceptors that control the secretion of ADH.

There is also controversy as to the existence of osmoreceptors in the peripheral nervous system. One area which has been implicated as a source for osmoreceptors is the liver (Haberich, 1968; Tordoff, Schulkin and Friedman, 1986, 1987). However, the existence and location of peripheral osmoreceptors are not known in much detail.

Extracellular Mechanisms

Three stimuli are believed to be critical for the regulation of extracellular fluids. First, detection of extracellular deficits are thought to originate in receptors sensitive to stretch of the atria, great veins in the pulmonary circulation, the aorta and the carotid arteries. These baroreceptors send vagal afferents to the supraoptic nucleus of the brain and trigger the release of ADH. Second, decreased blood flow through the kidneys can stimulate receptors within the kidneys to release renin. Third, the direct action of angiotensin II (AII) on the brain can contribute to extracellular thirst.

Stricker (1966) found that injections of PEG into rats whose kidneys had been removed resulted in a withdrawal of fluid from the extracellular compartment which produced a decrease in blood pressure. The ensuing hypovolemia stimulated the baroreceptors, but there was no release of renin because the kidneys had been removed. Nevertheless, the rats drank even though AII could not be formed, indicating that baroreceptor stimulation alone can induce drinking.

At approximately the same time, Fitzsimons (1964) showed that receptors in the kidneys could alone initiate drinking. When the flow of blood to the renal arteries was restricted by partially constricting the abdominal aorta above the renal arteries, the rats would drink. Yet, constrictions below the renal arteries which permitted normal renal perfusion pressure did not elicit drinking. Therefore, the studies of Stricker (1966) and Fitzsimons (1964) showed that both baroreceptors and renal blood flow detectors can independently stimulate hypovolemic drinking. However, the full behavioral effect may not be exhibited unless there is a synergy between the baroreceptors (with ADH release) and the angiotensin system (Epstein, 1982, 1983; Fluharty and Epstein, 1983; Rowland, 1980).

Drinking

In 1937, Gilman showed that cellular dehydration was an effective stimulus for thirst. In dogs, after iv administration of NaCl (which is confined to the extracellular space) a rapid and copious amount of drinking ensued, while urea (which can readily enter the cells) was not dipsogenic.

Many years later in 1961, Fitzsimons established that hypovolemia (extracellular fluid depletion) was a second and independent stimulus for eliciting thirst. Procedures such as hemorrhage, administration of hyperoncotic colloids and ligation of the inferior vena cava (Fitzsimons, 1964), all elicited drinking by decreasing blood volume without causing intracellular dehydration (i.e., not withdrawing water from the cells).

Two mechanisms exist for replenishing lost body water: (a) to drink water and (b) to get water from the foods eaten and from oxidation of foods.

Humans and animals seek out fluids when dehydrated. If fluid loss is primarily from the intracellular fluid compartment, water is the fluid of choice. However, if the fluid loss is primarily extracellular, both water and salt solutions may be consumed. After 24-hour water deprivation in humans, there was a significant shift toward feelings of being "very thirsty" when asked to rate their thirst levels on a visual analogue rating scale. In addition, the subjects reported that the taste of water shifted to "very pleasant" after 24-hour water deprivation (Rolls and Rolls, 1982). In animal studies, Denton (1982) showed that dehydrated sheep would consume both water and sodium chloride (NaCl) solutions and that the animals learned to press a bar and work for access to fluids.

Drinking can be classified as a highly motivated behavior (Toates, 1986). For instance, after intracranial angiotensin II administration, rats will either press a bar numerous times for a reward of only 0.1 ml of water or will drink quinine adulterated water (Rolls, Jones, and Fallows, 1972). After intracranial administration of AII, sleeping rats will awaken to drink, and under the same conditions rats that have been food-deprived for two days will stop eating and start drinking (Epstein, Fitzsimons and Rolls, 1970). In an instrumental paradigm, when the cost of gaining access to water is increased, the rats' strategy shifted to one of decreasing the total number of drinking bouts while simultaneously increasing the size of each drink (Marwine and Collier, 1979).

Sodium deprivation can induce animals to seek out salt solutions (Wolf, Schulkin and Simson, 1984). Kreickhaus (1970), using an instrumental task, taught thirsty rats to run to one arm of a T maze for distilled water and to the other arm for hypertonic NaCl. After the training procedure was completed, the rats were either water or Na^+ depleted and were found to run more quickly to the appropriate arm for fluid reward. In a more natural setting, there are reports of elk traveling for miles to the nearest salt licks. On approaching the licks, the elk would run to the salt spring and begin to drink the brackish water immediately. Similarly, herds of reindeer in the Arctic have been reported to drink sea water. Many other types of animals such as cows, pigs, porcupines, monkeys, elephants, sheep and rabbits have been reported to travel many miles and/or tolerate adverse conditions to reach either salt licks or brackish ponds (see Denton, 1982 for extended discussion). Thus, fluid consumption is one process by which hydromineral homeostasis is preserved.

The Renin-Angiotensin-Aldosterone System (RAAS)

As previously mentioned, animals can compensate behaviorally for body fluid losses, however, running to drink water at the smallest detectable decline in hydration is impractical, if not impossible. Thus, physiological mechanisms have evolved to help in the retention of water and minerals. A physiological mechanism involved in maintaining hydromineral homeostasis is related to activation of the RAAS.

Components of the RAAS are as follows: Renin is formed and stored in granules in specialized vascular smooth muscle cells of the kidney, the juxtaglomerular cells. Stimuli such as reduction in the concentration of sodium in plasma, reduced stretch of the juxtaglomerular cells associated with decreased blood pressure in the afferent arterioles, and either stimulation of the renal sympathetic nerves or increases in the concentration of epinephrine and norepinephrine in plasma, via activation of B-adrenoreceptors on juxtaglomerular cells, can mediate the release of renin. In response to any one or a combination of these stimuli, renin is released from the juxtaglomerular cells and cleaves angiotensinogen (an a_2-globulin) to form the decapeptide angiotensin I (AI). Angiotensin I, in the presence of angiotensin converting enzyme (ACE or kininase II), is first cleaved to angiotensin II (AII) in the endothelium of the vasculature and particularly by the pulmonary vasculature (Ng and Vane, 1967; Biron, Campeau and David, 1969). Angiotensin II may be further cleaved to angiotensin III (AIII). Physiologically, AII can induce release of antidiuretic hormone from the posterior pituitary gland (ADH, or vasopressin, is a peptide hormone which functions to increase water permeability and conserve water along the entire collecting duct and the last part of the distal tubule; ADH also has potent vasoconstrictor properties). Angiotensin II can also induce release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland as well as, sodium retention via release of aldosterone. It also has potent pressor properties. Angiotensin II may also act on sympathetic nerve terminals to enhance release of NE. More recently AII has been shown to act directly on the brain to stimulate the intake of water and NaCl (Epstein, 1982; Epstein, Fitzsimons and Rolls, 1970). A role for AIII has been implicated in the induction of water and NaCl intakes, and secretion of aldosterone from the adrenal cortex (See Figure 1-1 for diagrammatic representation).

Aldosterone, the final component of the RAAS, is a steroid hormone released from the adrenal cortex by AII, AIII, low plasma Na^+ concentration, high plasma K^+ concentration, or a fall in blood pressure. Aldosterone's site of action is the distal tubule where it stimulates Na^+ reabsorption and K^+ secretion (Na^+ reabsorption is accompanied by water reabsorption). In addition, aldosterone stimulates a Na^+ appetite which helps regain lost Na^+. In general, any situation in which conservation of Na^+ is necessary will stimulate release of aldosterone (Duling, 1983b; Carlson, 1986).

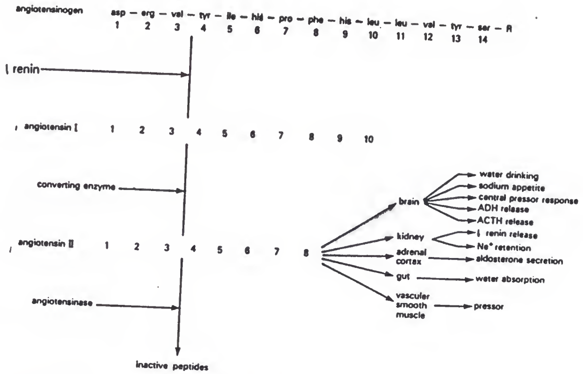

Figure 1-1: From top to bottom: **The synthetic cascade for production and degradation of angiotensin II, with the principal target organs and actions of the hormone shown at the right.** Reproduced from Epstein, A. N. (1983). The neuropsychology of drinking behavior. In Satinoff, E. and Teitelbaum, P. (Eds.) *Handbook of Behavioral Neurobiology, 6: Motivation*. New York, Plenum.

In summary, renin is released when water or Na^+ needs to be conserved. Renin converts angiotensinogen into AI, which is in turn cleaved to AII. Angiotensin II then helps restore plasma volume and Na^+ concentration by:
(a) inducing release of ADH, which conserves water along the distal tubule and collecting duct;
(b) inducing release of ACTH, which stimulates the adrenal cortex to release cortisol (or corticosterone);
(c) inducing systemic vasoconstriction to increase blood pressure;
(d) inducing release of aldosterone which is important for reabsorption of Na^+ and secretion of K^+;
(e) acting directly on the brain to induce water and Na^+ consumption.

All of the above effects are directed toward expansion of the extracellular fluid volume and elevation of blood pressure. Therefore, quite small deficits in body fluids can be compensated for initially by activation of the RAAS. Because of this pivotal role, it is not surprising to find that renin is widely distributed in terrestrial animals.

Renin is believed to have first appeared during early evolution of the osteichthyes (bony fishes) (Sokabe, Pang and Gorbman, 1978). Renin has not been identified in the kidneys of either elasmobranchs (cartilaginous fish) or cyclostomes. The juxtaglomerular apparatus in non-mammalian vertebrates is incomplete. [The juxtaglomerular apparatus is composed of the macula densa (which are specialized epithelial cells of the nephron distal tubule) and the juxtaglomerular cells (which are modified smooth muscle cells located on the afferent and efferent arterioles of most mammals)]. However, primitive bony fishes, teleosts, lung fishes, amphibians and reptiles have been found to have granulated epithelioid cells which resemble the mammalian juxtaglomerular cells (Nishimura and Ogawa, 1973). Macula densa cells are first clearly found in avians (Nishimura, 1978). Aldosterone was first found in the blood of bony fishes (Phillips, Holmes and Bondy, 1959; Denton, 1965). Therefore, the RAAS appears to have become functional only as evolution moved animals further from their watery environment. Development of the RAAS has enabled animals to abstain from visiting their water source until environmental conditions are more favorable and allow the animal greater freedom to travel longer distances away from its' source of water. However, as fluid deficits become too great, physiological mechanisms alone may not be able to compensate entirely for the deficit and behavioral mechanisms involving fluid consumption may be employed.

Mechanism of Action of the RAAS

It is currently hypothesized that AII, which is produced peripherally, may be acting both peripherally and centrally to induce drinking. Peripherally generated AII may be acting directly on angiotensin receptors in the adrenal cortex to induce aldosterone release. However, peripherally generated AII may also be acting at sites within the central nervous system. For years there has been debate whether peripheral AII can get into the central nervous system. However, recent studies have shown that there are areas of the brain where the blood-brain barrier is leaky (Simpson, 1981). These areas are collectively known as the circumventricular organs (CVOs) and include the subfornical organ (SFO), the organum vasculosum lamina terminalis (OVLT) and the area postrema (AP).

Direct action of AII in the brain was first reported by Booth (1968). Subsequently Epstein, Fitzsimons and Rolls, (1970), Severs et al., (1970) and Swanson and Sharpe (1973) confirmed that AII could elicit copious drinking with a short latency when injected directly into the brain. Epstein and colleagues (1970) also showed that the drinking behavior was quite specific since no other behaviors such as hyperactivity, eating or gnawing were observed after AII administration. Additionally, many other agents such as ADH, aldosterone, bradykinin etc. were tested and found to be nondipsogenic (Epstein, Fitzsimons and Rolls, 1970) while renin, AI and various fragments of AII were all highly dipsogenic when injected into the brain (Fitzsimons, 1971).

When injected with AII into either the SFO or preoptic area water satiated rats drank copiously. However, after lesioning the SFO, these rats no longer drank in response to administration of AII into the preoptic area. Control lesions made in adjacent tissue had no effect on drinking to administration of AII (Simpson and Routtenberg, 1973).

Prior administration of saralasin (AII antagonist) into the SFO decreased the pressor effect of subsequently administered AII (Mangiapane and Simpson, 1980). With injections of AII into the SFO, the pressor response occurred before the onset of drinking and always outlasted drinking. The threshold pressor dose was 10^{-16} moles AII, while the dipsogenic dose was between 10^{-14} and 10^{-13} moles. Therefore, at very low doses of AII into the SFO, an increase in arterial pressure occurred in the absence of drinking. In order to elicit drinking from sites adjacent to the SFO (such as the dorsal third ventricle and ventral fornical commissure), doses one to two orders of magnitude larger than those utilized for the SFO had to be used. Injections of AII into the dorsal third ventricle elicited significantly less pressor and drinking activity than injections into the SFO (Magiapane and Simpson, 1980). Thus, Simpson and Routtenberg (1973) hypothesized that blood-borne AII acts at the SFO to induce drinking.

Simpson, Epstein and Camardo (1978) also showed that AII, injected directly into the SFO, could elicit drinking while saralasin, a specific competitive inhibitor of AII, infused into the SFO, suppressed the drinking response. After the termination of the infusion, the animals again drank to iv administration of AII. Ablation of the SFO resulted in selective and permanent decreases in drinking elicited by iv administration of AII. Additionally ablations of the SFO resulted in reduced drinking to isoproterenol (B-adrenergic agonist) and PEG. Therefore, specific and reversible blockade of receptors for AII in the SFO decreased drinking to administration of AII.

Angiotensin II, applied microiontophoretically to brain slices of rat (Felix and Phillips, 1979; Knowles and Phillips, 1980) and cat (Phillips and Felix, 1976), produced a marked excitation of cells within both the SFO and OVLT and these responses were inhibited by application of saralasin.

Fitzsimons, Epstein and Johnson, (1978) found that pepstatin (an inhibitor of renin), when injected into the anterior cerebral ventricles, inhibited drinking following administration of renin. Intracranial renin and AI induced drinking were both inhibited by prior intracranial administration of SQ 20881 (an angiotensin converting enzyme inhibitor, ACEI).

In addition to the behavioral and electrophysiological data, there have been numerous reports of degrees of specific ^{125}I-AII binding in the CVOs of rats (Bennett and Snyder, 1976; Harding, Stone and Wright, 1981; Sirett et al., 1977). Receptors for AII have also been located in the AP of the dog (Gildenberg, Ferrario and McCubbin, 1973; Joy and Lowe, 1970). However, Buggy et al., (1975) and Fitts and Masson (1989) propose that the AP is not an important dipsogenic site in the rat.

Miselis, Shapiro and Hand (1979) using a variety of anatomical techniques have shown that neurons in the SFO send axons to the OVLT, the supraoptic nucleus and the nucleus medianus of the medial preoptic area. Camacho and Phillips (1981) injected horseradish peroxidase (HRP) directly into the OVLT and found reciprocal projections to cell bodies in the SFO. The anatomical results may explain why lesions of the median preoptic nucleus abolished drinking stimulated by angiotensin (Shrager and Johnson, 1980). Also knife cuts between the SFO and the median preoptic nucleus abolished drinking in response to administration of angiotensin (Lind and Johnson, 1982). Therefore, blood-borne AII may stimulate neurons in the SFO which, in turn, send their axons to the median preoptic nucleus. These axons have been shown, by immunocytochemical methods, to contain angiotensin.

In addition to this "angiotensinergic" pathway from the SFO to the median preoptic nucleus, two other angiotensin-sensitive pathways project from the SFO to the midbrain (Swanson, Kucharczyk and Mogenson, 1978). The first pathway descends through the medial forebrain bundle and terminates in the lateral hypothalamus, the mammillary body, the ventral tegmental nucleus and the ventral tegmental area (which may explain how water can be rewarding, since the ventral tegmental area has been linked to reinforcement and reward systems; see Carlson 1986 for review). Swanson and colleagues (1978) suggest that this pathway primarily mediates the effects of angiotensin on drinking. The second angiotensin-sensitive pathway terminates in the midbrain central gray and appears to mediate the effects of angiotensin on regulation of blood pressure (Swanson, Kucharczyk and Mogenson, 1978).

Therefore, activation of the RAAS, in addition to its physiological effects, is often associated with the behavioral phenomena of increasing water and NaCl intake.

History of Isoproterenol Induced Drinking

In 1967 Lehr, Mallow and Krukowski were the first investigators to report that peripheral injections of isoproterenol, a mixed B_1- and B_2-adrenergic agonist, induced thirst in rats, and that thirst-related neurons within the central nervous system may be involved. Soon thereafter, Leibowitz (1971a, 1971b) reported that B-adrenergic stimulation by intrahypothalamic administration of isoproterenol (40 ug) could elicit drinking while a-adrenergic stimulation could inhibit drinking. The results led Leibowitz to propose that both eating and drinking were reciprocally controlled by activation of the central nervous system via a- and B-adrenergic receptors. However, the central dose of isoproterenol (40 ug) used by Leibowitz to induce thirst was extremely high, especially in light of the fact that Lehr and colleagues (1967) could induce drinking with as little as 5 ug isoproterenol administered peripherally. Therefore, it was possible that the central injections of isoproterenol used by Leibowitz were leaking into the periphery to have their dipsogenic effects. Following up on the work of Lehr, Mallow and

Krukowski (1967) and Leibowitz (1971a, 1971b), Fisher and colleagues performed numerous studies to determine the central effects of isoproterenol (see Fisher, 1973 for complete review). To determine whether centrally injected isoproterenol was having its dipsogenic effect through the peripheral nervous system, Fisher injected tritiated isoproterenol (25 ug) into either the hypothalamus or hippocampus and took peripheral blood samples at both 30 and 60 minutes post-injection. The results showed that there was, in fact, radioactive leakage to the peripheral nervous system after central nervous system injections of isoproterenol. Thus, Fisher concluded that centrally injected isoproterenol was dipsogenic because of leakage to the peripheral nervous system (Fisher, 1973). In the peripheral nervous system isoproterenol is known to bind to receptors located on the kidney (to induce the release of renin) and to receptors located on the heart (to increase heart rate; Berne and Levy, 1983b; Lefkowitz, 1974).

For years it has been debated whether stimulation of B-adrenergic receptors works via activation of the RAAS. Some investigators hypothesize that there is complete dependence of B-adrenergic drinking on the RAAS (Fregly, Fater and Greenleaf, 1982; Houpt and Epstein, 1971) while other investigators argue that the RAAS plays only a permissive role in initiating thirst (Hosutt, Rowland and

Stricker, 1978; Stricker, 1978). The controversy on the role of the RAAS in B-adrenergic drinking has still not been resolved.

History of Strain Differences in NaCl Intake

It has long been known that there are substantial differences between strains of rats in the regulation of hydromineral balance (Walsh, 1980) and blood pressure (Dahl, Heine and Tassinari 1962; Hall, Ayachi and Hall, 1976; Okamoto et al., 1972; Oparil et al., 1988). Hypertension has been known to occur spontaneously in colonies of rats for a number of years (Medoff and Bongiovanni, 1945). However, one of the pioneers to capitalize first on the spontaneous occurrence of hypertension in a population of rats was Dahl (Dahl, Heine and Tassinari, 1962). Dahl showed that by selectively inbreeding Sprague-Dawley (SD) rats which: (a) spontaneously exhibited hypertension, and (b) which appeared to be resistant to hypertension, he could differentiate substrains of rats. Dahl named the substrain in which salt hypertension could easily be induced the "sensitive" strain, while those in which it was difficult, if not impossible, to elicit NaCl related hypertension the "resistant" strain (Dahl, Heine and Tassinari, 1962). Dahl and colleagues further showed that these substrains displayed different susceptibilities to other forms of experimentally induced hypertension such as unilateral renal artery constriction (Dahl, Heine and Tassinari, 1963), deoxycorticosterone acetate (DOCA)-salt (Dahl, Heine and

Tassinari, 1963) and cortisone treatment (Dahl, Heine and Tassinari, 1965). Thus, the results were not an anomaly of salt-induced hypertension alone, but instead represented a true resistance or susceptibility to all forms of hypertension.

At approximately the same time that Dahl and his coworkers were testing their "resistant" and "sensitive" strains of rats, Okamoto and Aoki (as cited in Okamoto et al., 1972), working in Japan, were able to breed selectively a strain of rats which became hypertensive spontaneously. Together, the work of Dahl and colleagues, as well as Okamoto and coworkers, have shown the importance of genetic factors in either the development or resistance of hypertension.

Fischer 344 Rats

Classically, the Fischer 344 (F344) strain of rats has been used in research on aging and tumor growth (Avakian, Horvath and Colburn, 1984; Chiueh, Nespor and Rapoport, 1980; Gambert and Barboriak, 1982). However, in 1972 Molteni and Brownie noted that the F344 rats, a strain not specifically inbred for cardiovascular traits, unexpectedly showed only a minimal increase in blood pressure after unilateral nephrectomy and 8 weeks of continuous access to 1% NaCl solution. This result was in contrast to the SD strain which developed marked hypertension, (systolic blood pressure of 150 mm Hg or greater), as well as renal and cardiovascular lesions after similar treatment. Hall, Ayachi and Hall (1976) reported that F344 rats naturally display a low intake of 1% NaCl solution. However, if F344 rats were unilaterally nephrectomized and given access to a 1% NaCl + 5% sucrose solution, they would consume an average of 75-126 ml/day of the saline-sucrose solution. These saline-sucrose intakes were up to 400-600% over baseline water intakes. Although the blood pressure of the F344 rats given the saline-sucrose solution did increase above that of F344 rats given only water, a systolic blood pressure greater than 134 mm Hg was never reported during the 6-month study. Thus, the F344 rats appeared to have an immunity to salt-induced hypertension.

Further behavioral studies by Midkiff et al. (1985, 1987) have shown that the F344 rats do not show a spontaneous preference for either hypotonic or isotonic NaCl solutions. This differs from other strains such as Wistar and SD rats that consume large amounts of both hypotonic and isotonic NaCl when given a choice between NaCl and water. In addition to the F344 rats, Midkiff et al. (1985) studied two other inbred strains of rats, the Munich-Wistar and the Buffalo strains, for salt preference. They found that these two strains showed Na^+ preferences similar to the outbred Wistar strain. The preference aversion curves for the Wistar, Munich-Wistar and Buffalo rats revealed that hypotonic NaCl solutions were preferred relative to water while hypertonic NaCl solutions were avoided relative to water, thus generating the typical inverted U-shaped preference aversion curve which has been characterized for numerous other strains of rats (Fregly and Rowland, 1985). These results were in sharp contrast to the F344 rats which did not prefer any concentration of NaCl tested. The decreased preference for NaCl in the F344 rats was not due to a general abnormality of the taste system since F344 rats showed preference aversion curves for the remaining three modalities of taste (sweet, sour and bitter) similar to those of the other strains (Midkiff et al., 1985).

Midkiff et al. (1985) studied salt-preference as opposed to salt-appetite. The distinction between salt-appetite and salt-preference is crucial. Ingestion of salt in response to a known salt depletion constitutes a salt appetite. In contrast, the rats from Midkiff's study were maintained on a laboratory diet which contained Na^+ levels in excess of amounts necessary for normal growth; thus a salt-preference was tested. Additional work by Midkiff et al. (1987) was undertaken to determine whether F344 rats would show a salt-appetite when made Na^+ deficient. Midkiff and colleagues (1987) showed that after administration of the diuretic furosemide, the F344 rats would show a Na^+ appetite, but the Na^+ appetite was small when compared to Wistar rats subjected to the same paradigm. However, the decreased Na^+ consumption in F344 rats was not due to decreased Na^+ loss since analysis of the amount of urinary Na^+ after treatment with furosemide revealed no significant difference between Wistar and F344 rats. In agreement with previous work, there was no difference between the F344 and Wistar strains in intake of water following administration of furosemide. Also, an analysis of water balance after treatment with furosemide revealed no significant differences between the F344 and Wistar strains, nor was the percent change in body weight different. Thus, the F344 rats differed from the Wistar rats only in their consumption of NaCl.

To determine whether the avoidance of NaCl by the F344 strain was due to oral factors versus postingestive factors, Grill and Bernstein (1988) utilized a test of taste-reactivity. The advantage of this type of analysis is that it allows for testing of the animal's oral behaviors immediately after infusion of a chemical stimulus, prior to any postingestive consequences. Typically the tests of taste-reactivity are completed in 60 seconds and Grill and colleagues (Berridge et al., 1984) have been able to categorize the behaviors into either aversive or ingestive responses. When tested in the Na^+ replete state, there were significant differences between the Wistar and F344 strains to infusions of NaCl solution. The authors noted that at every concentration of NaCl tested, the F344 rats had significantly lower ingestive scores and higher aversive scores than the Wistar rats. Therefore, the palatability of NaCl solutions appears to be lower in F344 rats. However, while Na^+ replete, there were no significant differences between the strains in taste reactivity to quinine solution and there were no significant differences between the strains for ingestive scores, aversive scores or aversive response-latency after quinine. Grill and Bernstein then repeated their test of taste-reactivity after depleting the Wistar and F344 rats of Na^+ by means of furosemide. Generally, the authors found significant shifts in taste-reactivity for NaCl for both strains of rats. These shifts were characterized by increased ingestive responding and decreased aversive responding when compared to the Na^+ replete condition. After rendering the F344 rats Na^+ deficient, there were increased ingestive and decreased aversive scores for each NaCl concentration tested. For the Wistar strains, Na^+ depletion enhanced the ingestive scores for all the Na^+ concentrations examined, but did not affect the already low aversive scores. When 24-hour salt intakes were examined after treatment with furosemide in both strains, an increased preference for NaCl was apparent. Both F344 and Wistar strains increased their intake of 0.3 M NaCl after treatment with furosemide. Thus, administration of furosemide essentially reversed the relative NaCl aversion found in replete F344 rats. This shift to decreased aversive scores and increased ingestive scores after Na^+ deficiency has also been reported for the SD strain of rats (Berridge et al., 1984).

Recent work by Rowland and Fregly (1988a) has confirmed the lack of spontaneous preference for NaCl solutions in F344 rats and expanded the conditions under which F344 rats would elicit an appetite for salt (For a review of factors known to induce an appetite for NaCl see Fregly and Rowland, 1985). The authors determined that adrenalectomy would induce an appetite for NaCl in F344 rats, as would administration of high doses of the mineralocorticoid, deoxycorticosterone acetate (DOCA) (Fregly and Waters, 1966; Rice and Richter, 1943; Tosteson et al., 1951; Wolf, 1965). When F344 rats were adrenalectomized and given graded doses of DOCA, reversal of the appetite for NaCl occurred. This is similar to the results obtained in adrenalectomized SD rats given replacement doses of DOCA (Clark and Clausen, 1943). Thus, F344 and SD rats are comparable in their responses to mineralocorticoids.

Rowland and Fregly (1988c) also studied various challenges to body fluid homeostasis in F344 rats. When water was mixed with dry chow to form a wet mash-mixture, the F344 rats decreased their spontaneous water intake as the amount of water in the mash-mixture increased. At the highest ratio of dry chow:water (1:1.6), the F344 rats consumed negligible amounts of drinking water (0.2 ml/24 hours). In contrast, SD rats still showed a mean intake of 16.2 ml/24 hours and were grossly overhydrated. Thus, under this experimental paradigm, the F344 rats apparently regulated their water intake more precisely than SD rats.

Rowland and Fregly have also studied NaCl intake after administration of furosemide and placement on Na^+ deficient diet (1988a). Rowland and Fregly, again replicating the work of Midkiff et al. (1987), found that they could elicit a NaCl appetite in F344 rats. They also found a NaCl appetite in F344 rats after activation of the RAAS. Previous work by Fregly (1980) has shown that chronic administration of the angiotensin converting enzyme inhibitors (ACEIs), such as captopril, result in increased intake of both isotonic and hypertonic NaCl solutions in SD rats. Based upon this information, Rowland and Fregly chronically administered ACEIs to induce an appetite for NaCl in F344 rats. In addition to the ACEI captopril, the authors tested the more potent ACEIs, enalapril (MK421) and ramipril (Hoe 498), which have also been shown to induce an appetite for NaCl in SD rats (Rowland and Fregly, 1988b). They found that all three ACEIs, when added to the diet, produced an appetite for NaCl in the F344 rats. They also reported that the increased intake of NaCl was related to the increased duration of the treatment since removal of ACEIs from the food resulted in a return to baseline intakes of NaCl within 2-3 days. Another parameter often used to study the state of the RAAS is plasma renin activity (PRA). Rowland and Fregly found that there are no significant differences in PRA between F344 and SD rats under basal conditions (Rowland and Fregly 1988d). To confirm that the RAAS was affected by treatment with ACEIs, PRA was measured in F344 rats and found to be increased by 290% after 2 days of treatment with captopril. Therefore, there is a very rapid change in the RAAS after treatment with ACEIs in the

F344 rats. These results are in agreement with the results from SD rats tested under the same paradigm. In addition, when enalapril was administered by minipump to F344 rats, intake of NaCl increased from a baseline of 1 ml/day to 41 ml/day after 6 days of treatment with enalapril. Thus, the increased appetite for NaCl was not merely due to an increased palatability of the food due to the presence of the ACEIs in the diet.

Rowland and Fregly calculated that in all of the above mentioned paradigms, the F344 rats drank an amount of NaCl appropriate to their Na^+ loss. However, their intakes were less than those of the Wistar and SD strains under similar conditions. For example, the F344 rats lost approximately 0.9 mEq of Na^+ after treatment with furosemide while they consumed approximately 1.2 mEq of Na^+ when allowed access to NaCl solutions (Rowland and Fregly, 1988a). Similarly, Midkiff et al., (1987) found that the F344 rats drank only 20-40% as much NaCl as comparably treated Wistar rats when Na^+ depletion was induced with furosemide. All of the above information has led Rowland and Fregly (1988c) to suggest that the F344 rats may be "internal" rather than "external" regulators, extending Kanter's (1953) original use of this term to explain individual differences in drinking of dogs.

The previous work of Rowland and Fregly (1988a) showed that administration of ACEIs resulted in decreased drinking in the F344 when compared with the SD strains. This suggested there may have been differential activation of the

RAAS in the two strains. Rowland and Fregly (1988c), therefore, injected a dipsogenic dose of AII subcutaneously (sc) and allowed F344 rats access to either 0.15 M NaCl or distilled water or a choice between water and 0.15 M NaCl. The authors found that after sc administration of AII, the F344 rats drank an amount of water comparable to that shown by SD rats after the same dose of AII. When given 0.15 M NaCl as the test solution, the F344 rats drank increased amounts of NaCl, again comparable to SD rats. However, when the F344 rats were given a choice between water and 0.15 M NaCl, they showed no preference for NaCl over water, in contrast to the SD rats. In addition, when F344 rats were implanted with jugular catheters and infused with AII for 1 hour and allowed access to distilled water, 0.15 M NaCl or a choice between the two, the F344 rats consumed water and NaCl solutions similar to those of SD rats in a single bottle test paradigm. When two bottle tests were given, once again no preference for NaCl over water was found in F344 rats. Also when the F344 rats were infused with hypertonic NaCl and given access to water, the amount of water consumed was comparable to the amount ingested by SD rats. However, after administration of PEG, the F344 rats drank less than SD rats given PEG. Therefore, the behavioral response of F344 rats to AII, both sc and intravenously (iv), as well as hypertonic NaCl, are comparable to the behavioral responses of SD rats, while the behavioral response to administration of PEG is decreased in

F344 rats. Thus, there is a paradox. F344 rats, when compared to SD rats, drink equal amounts of either water or 0.15 M NaCl in response to exogenous administration of AII and DOCA, and they appear to generate normal levels of renin as measured by PRA. Yet, the F344 rats have decreased salt-appetite and salt-preference to various stimuli which induce copious salt intakes in other strains of rats such as the SD and Wistars.

Since activation of the RAAS is known to induce an appetite for salt (Fregly and Rowland, 1985) and since the F344 rats do not show a salt preference, the present experiments were designed to explore the interactions between the RAAS and the cardiovascular system of F344 rats compared with outbred rats of the SD strain.

CHAPTER 2

BEHAVIORAL STUDIES

The purpose of the following studies is to determine whether there may be decreased activation of the RAAS in F344 rats. Since F344 rats drink less after administration of PEG (Rowland and Fregly, 1988c), the first experiment was designed to examine the behavioral response of F344 rats to administration of isoproterenol, which is also known to activate the RAAS (Leenen and McDonald, 1974). The second experiment was designed to test the generalizability of the behavioral response to other stimuli known to work through activation of the RAAS. The third and fourth experiments systematically examine the behavioral responses of F344 rats when compared to SD rats after administration of various components of the RAAS, both peripherally and centrally.

General Methods

Animals and Housing

Male F344 rats, initially weighing 150-200 g, were obtained from Hilltop Farms, Scottdale, PA. Sprague-Dawley (SD) rats, also 150-200 g, were obtained from Zivic Miller Labs, Allison Park, PA. In later experiments animals of both strains were obtained from Harlan Sprague-Dawley, Indianapolis, IN. SD rats grow at a much faster rate, and attain final body weights greater than F344 rats. Therefore, throughout the following studies, the SD rats, although of similar body weight, were approximately 2-3 weeks younger (although still young adults) than the F344 rats. This allowed for rats of both strains to be of comparable body weights for the testing of drug effects on behavior. Upon arrival, all rats from both groups were given a minimum of 7 days adaptation to the laboratory. All animals were singly housed in stainless steel hanging wire cages. Purina Laboratory Chow #5001 was available in a hopper on the front of the cage and tap water was available from a plastic bottle with a metal spout. Both food and water were available ad libitum except during testing sessions. The colony was kept on a 12:12 hr light/dark cycle (lights on 0600-1800 hr) and temperature was maintained at $24 \pm 2^{\circ}$ C. Upon arrival in the colony, all animals were handled extensively (2-3 times/day) to minimize stress during the testing procedures.

Drugs

All angiotensins were prepared and stored in glassware coated with SigmaCote, since AIII is known to adhere to a greater degree than AII to borosilicate glass and plastics. The adhesion is due to a net positive charge carried by AIII (Wright et al., 1985). Throughout the study, all doses of angiotensin were based on the ratios of the molecular weights of Angiotensin I (AI), AII, and Angiotensin III (AIII). By basing dosages on the molecular weight, the relative potencies of the drugs can be compared on a molar basis. However, because the half-life of ^{125}I-AIII is shorter than the half-life of ^{125}I-AII (14 versus 28 seconds; Wright et al., 1985), we found that a three times higher molar dosage of AIII was necessary to elicit a drinking response in our animals. Angiotensin I-acetate salt, human sequence [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu]; AII-acetate salt, human sequence [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe] ; and AIII [Des-Asp1-Angiotensin II]-acetate salt; (Arg-Val-Tyr-Ile-His-Pro-Phe) were obtained from Sigma Chemical Co (catalog numbers A-9650; A-9525; A-0903 respectively). Isoproterenol was the hydrochloride salt of the d,l racemate (Sigma #I-5627), and doses are expressed as this salt. Serotonin (5-hydroxytryptamine) was the creatinine sulfate complex (Sigma #H-7752). All other chemicals were reagent grade from Fisher Scientific.

Statistics

Data were analyzed using an Ed Sci statistics program for t-tests. Between/within ANOVA or ANCOVA were from an ANOVA II version 1.1 General Analysis of Variance for an Apple II Plus with Newman-Keuls for post-hoc tests. Significance was set at a probability of $p<0.05$.

Experiment 1 A, B & C: Isoproterenol-Induced Thirst

Rationale

Isoproterenol is a B-adrenergic agonist which has been used extensively to study functioning of the B-adrenergic system (Leenen and McDonald, 1974; Fregly et al., 1983). Peripherally administered isoproterenol is dipsogenic and decreases peripheral resistance, thus inducing a quick, dose-related decrease in blood pressure which induces release of renin (Leenen and McDonald, 1974). Release of renin, in turn, leads to an increase in the circulating levels of AI and AII. AII then helps to stabilize blood pressure through its potent pressor actions. Therefore, administration of isoproterenol is also a procedure used to stimulate the RAAS (Leenen and McDonald, 1974; Fregly et al., 1983). Since F344 rats show no spontaneous preference for NaCl solutions, we were interested in determining whether the F344 rats would show either any anomalies or deficits in their behavioral responses when subjected to a challenge of the RAAS. Thus, the first study was designed to measure the dipsogenic effect of isoproterenol in F344 and SD rats. Due to the strain difference found in Experiment 1 A, follow-up studies 1 B and 1 C were performed to examine the generality of this phenomenon.

Methods

Procedure 1 A. Two doses of isoproterenol were tested for their dipsogenic effects in a cross-over design. During tests, food was removed from each cage and water intake was measured from calibrated burettes with metal spouts placed at the front of the cage. Water was presented at room temperature. Groups of F344 (n=6) and SD (n=6) rats were given isoproterenol, 25 ug/kg body weight sc, and allowed one hour of access to water during the middle of the light phase. Additional groups of F344 (n=6) and SD (n=6) rats were given isoproterenol, 100 ug/kg body weight sc, and allowed one hour of access to water. Leenen and McDonald (1974) have shown that near maximal drinking was attained with 100 ug isoproterenol/kg sc after 1 hour and this has been confirmed and expanded upon by Johnson et al., 1981. Similarly, Leenen and McDonald (1974) found that isoproterenol, at a dosage greater than 10 ug/kg sc, increased water consumption, while Johnson et al., (1981) reported that 30 ug isoproterenol/kg sc resulted in a significant increase in water consumption and rise in the concentration of AII in plasma. The mean body weight for the F344 group was 287 g, while the mean body weight for the SD group was 284 g.

Two days later the animals were treated with the other dose of isoproterenol and water intakes again measured for 1 hour.

Procedure 1 B. Rats are primarily nocturnal creatures. The results of Experiment 1 A showed significant differences in daytime water intakes between the F344 and SD rats. Therefore, Experiment 1 B was devised to determine whether there may have been any type of nycthemeral rhythm inhibiting water intake in the F344 rats during the light phase of the light/dark cycle (Carlson, 1986; Moore-Ede, 1986; Rowland, Bellush and Fregly, 1985).

Using different groups of rats, saline and isoproterenol (25 ug/kg sc) were administered to both strains during the dark phase of the day/night cycle and water intake recorded for 1 hr.

Procedure 1 C. Rowland and Fregly (1988c) have shown that after administration of AII, the intake of 0.15 M NaCl by F344 rats was comparable to the intakes by SD rats. Since the F344 rats consumed significantly less water than SD rats after administration of isoproterenol (Experiment 1 A), we were interested in determining: (a) whether the F344 rats could be induced to consume NaCl after administration of isoproterenol and (b) if the F344 rats did consume 0.15 M NaCl, were their intakes comparable to the intakes of SD rats.

Using the same group of rats as in Experiment 1 B, both strains were injected with saline and isoproterenol (25 ug/kg sc) and allowed access to 0.15 M NaCl for 1 hr during the middle of the light-cycle.

Results

Experiment 1 A. There was no significant effect of the order of administration of isoproterenol, so the results from the two studies were combined. Figure 2-1 shows the mean one-hour water intakes induced by the two doses of isoproterenol. The F344 rats drank significantly less than the SD rats at both the 25 and 100 ug/kg doses of isoproterenol. After administration of the 25 ug/kg dose, the F344 rats consumed 32% of that consumed by the SD rats, while at the 100 ug/kg dose, the F344 rats drank 55% when compared to their SD counterparts. The majority of drinking in both strains was complete by 30 minutes after injection. An ANOVA showed a significant main effect of strain, F(1,20)=10.1, p=0.004, but no significant main effects of dose or interactions.

Experiment 1 B. In the nocturnal study, the F344 rats again consumed significantly less water than SD rats, with a highly significant main effect of strain F(1,16)=29.0, p<0.001; drug F(1,16)=16.9, p=0.01 and a significant strain x drug interaction F(1,16)=11.0, p=0.04 (Figure 2-2).

Experiment 1 C. When given isoproterenol and access to 0.15 M NaCl as the test solution, the F344 rats consumed significantly less than the SD rats, with a main effect of strain F(1,9)=17.9, p=0.02 (Figure 2-3).

Discussion

The behavioral tests revealed striking differences between the F344 and SD rats in both water and NaCl intake after isoproterenol. Although fully-grown SD rats typically are larger than F344 rats, the present results have ruled out this confounding variable by using rats of slightly different ages but similar body weights. We have tested SD and F344 rats at various ages in other studies and have always seen the same effect. The hyporesponsiveness of F344 rats also cannot be ascribed to an unusual inhibitory effect of light on drinking (Moore-Ede, 1986; Rowland, Bellush and Fregly, 1985) because the effect persisted during the dark phase. Further, the decreased drinking in the F344 rats could not be due to dilutional inhibition (Blass and Hall, 1976; Stricker, 1969) since the decreased drinking was also found when 0.15 M NaCl was the test solution. In fact,

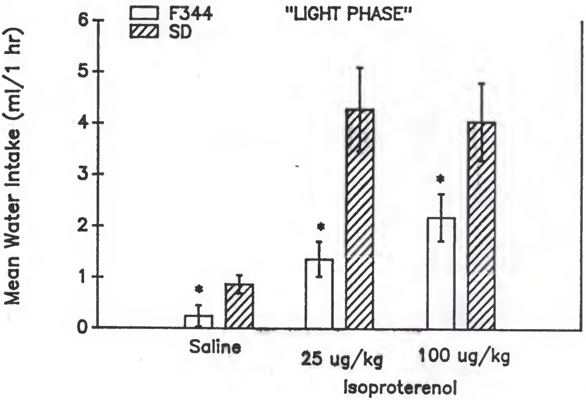

Figure 2-1: Mean (±S.E.M.) 1 hour water intakes of F344 and SD rats after administration of saline and isoproterenol (25 or 100 ug/kg) sc. during the light phase of the day/night cycle.

* $p<0.05$ relative to SD intakes.

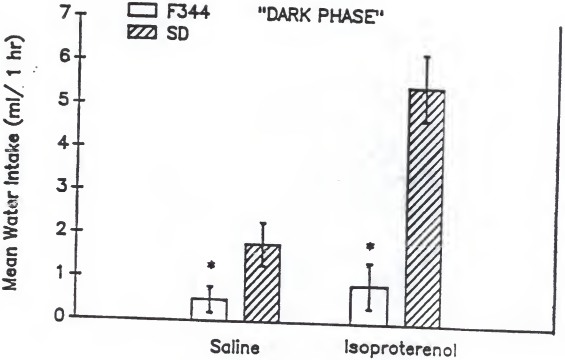

Figure 2-2: Mean (±S.E.M.) 1 hour water intakes of F344 and SD rats after administration of saline and isoproterenol (25 ug/kg) sc. during the dark phase ofthe day/night cycle.
* p<0.05 relative to SD intakes.

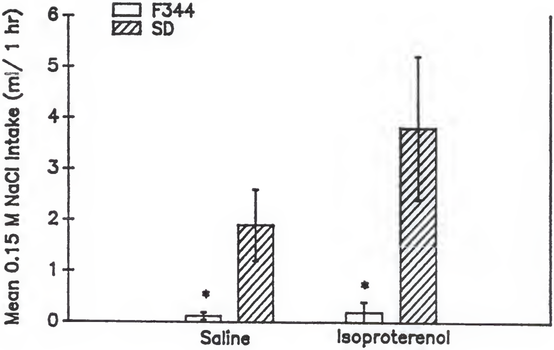

Figure 2-3: Mean (±S.E.M.) 1 hour 0.15 M NaCl intakes of F344 and SD rats after administration of saline and isoproterenol (25 ug/kg) sc. during the light phase of the day/night cycle.

* $p<0.05$ relative to SD intakes.

administration of isoproterenol was unable to elicit a NaCl appetite in F344 rats, which is in contrast to the NaCl appetite produced in this strain by administration of AII (Rowland and Fregly, 1988c). It thus appears that a constitutional, rather than an environmental, factor accounts for the marked differences in drinking between the two strains.

To test further for behavioral activation of the RAAS between the two strains, Experiments 2, 3 and 4 were designed to examine responsiveness of drinking to exogenous administration of serotonin (5HT; Experiment 2) and the angiotensins, both peripherally (Experiment 3) and centrally (Experiment 4).

Experiment 2: Serotonin Induced Thirst

Rationale

Serotonin (5HT; 5-hydroxytryptamine) is a potent vasodilator and dipsogen and is believed to induce drinking via activation of the RAAS (Meyer, Abele and Hertting, 1974). Thus, Experiment 2 was designed to determine whether the decreased drinking response of the F344 rats after administration of isoproterenol was due either to a specific hyporesponsiveness to isoproterenol or is generalizable to any drugs which have their mechanism of action through activation of the RAAS.

Procedure

Drinking tests were conducted in the middle of the light cycle with food and water removed. One-half of the animals from each strain (F344, n=8; SD, n=9) was injected with either 5HT (4 mg/kg) or saline sc and allowed 1 hr access to water from calibrated burettes placed at the front of the home cage. Forty-eight hours later the animals were again tested with the alternate injection.

Results

Figure 2-4 shows that the F344 rats consumed significantly less water than the SD rats after administration of 5HT (ANOVA main effect of strain F(1,15)=4.98, p=0.039). Thus, the hyporesponsiveness in the consumption of water by the F344 rats appears to be generalizable to activation of the RAAS and/or the induction of hypotension.

Experiment 3 A & B: Drinking to Peripheral Administration of AI, AII, and AIII

Procedure 3 A & B

Animals of both strains (n's=12) received a sc injection of either isotonic saline, AI (120 ug/kg), AII (100 ug/kg) or AIII (270 ug/kg) and, in separate tests, intakes of water and 0.15 M NaCl were tested for 1 hr. The doses of AI and AII are equimolar but, as mentioned above, we have found an equivalent dose of AIII (90 ug/kg) is ineffective as a dipsogen and so we have used three times this dose. Drinking tests were conducted in the middle of the light cycle with water and food removed from the cage. The test fluid was placed at the front of the home cage in

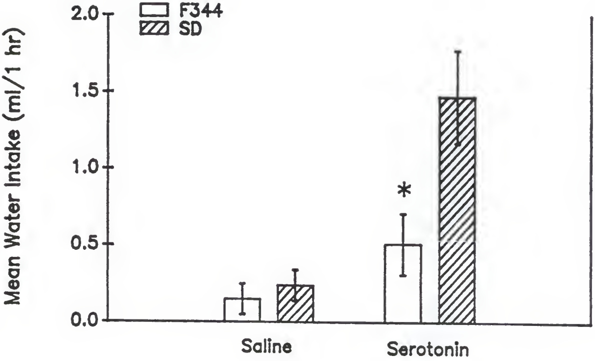

Figure 2-4: Mean (±S.E.M.) 1 hour water intakes of F344 and SD rats after administration of saline and serotonin (4 mg/kg) sc. during the light phase of the day/night cycle.
* p<0.05 relative to SD intakes.

calibrated burettes with stainless steel spouts and intake measured to the nearest 0.1 ml. A minimum of 72 hours elapsed between tests.

Results

Experiment 3 A. At one hour after peripheral administration of AI, AII and AIII (Figure 2-5), F344 rats drank significantly less water than their SD counterparts (ANCOVA significant main effect of strain $F(1,21)=8.1$, $p=0.009$, with saline intakes as covariate). Post-hoc analysis with Newman-Keuls revealed that the F344 rats drank significantly less water than SD rats after AI, $p<0.01$ and AIII, $p<0.05$, administration. There was no significant difference between the strains after administration of AII, in confirmation of the work of Rowland and Fregly, (1988c).

Experiment 3 B. NaCl intake after peripheral administration of AI, AII and AIII revealed that when rats were given 0.15 M NaCl as a test solution, the F344 rats drank significantly less (Figure 2-6) than SD rats, ANCOVA (saline intakes as covariate) main effect of strain $F(1,21)=10.5$, $p=0.004$. Again, Newman-Keuls results showed that the F344 rats drank significantly less after AI, $p<0,05$ and AIII, $p<0.01$. NaCl intake was similar between the two strains after AII. Of interest, is the fact that the F344 rats consumed 0.15 M NaCl in excess of their basal 24 hour intakes after administration of angiotensin. Thus, F344 rats can be

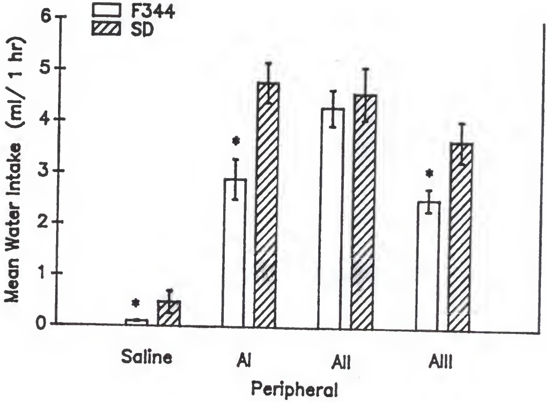

Figure 2-5: Mean (±S.E.M.) 1 hour water intakes of F344 and SD rats after administration of saline, AI (120 ug/kg), AII (100 ug/kg) or AIII (270 ug/kg) sc.
* p<0.05 relative to SD intakes.

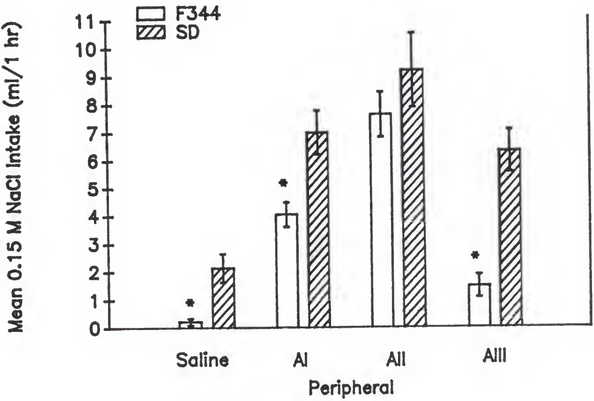

Figure 2-6: Mean (±S.E.M.) 1 hour 0.15 M NaCl intakes of F344 and SD rats after administration of saline, AI (120 ug/kg), AII (100 ug/kg) or AIII (270 ug/kg) sc.
* p<0.05 relative to SD intakes.

induced to consume quite large amounts of NaCl under certain circumstances.

Discussion

The results of Experiments 3A and 3B show that the F344 rats drink significantly less than SD rats after administration of AI, but not after administration of AII. This suggests that there may exist some type of peripheral deficit in angiotensin converting enzyme (ACE) activity in the F344 rats.

Experiment 4 A & B: Drinking to Central Administration of AI, AII and AIII

As mentioned in Chapter 1, it is currently hypothesized that peripherally generated AII may be acting at sites both in the peripheral nervous system and within the central nervous system (see Phillips, 1982 for review). The CVOs are sites within the central nervous system that possess a capillary network which lacks tight junctions. Thus, circulating AII can reach these CVOs and have central effects. In the rat, the most prominent CVOs implicated in the central actions of AII are the SFO and the OVLT. The area postrema (AP) is an important site of AII action in the dog (Gildenberg, Ferrario and McCubbin, 1973; Joy and Lowe, 1970), however, the AP does not appear to be as critical a site in the rat (Haywood et al., 1980). Both the SFO and OVLT have been found to contain receptors for AII (Harding, Stone and Wright, 1981; Sirett et al., 1977) and there appear to be direct neural connections between the SFO and OVLT (Camacho and Phillips, 1981; Miselis, Shapiro and Hand, 1979).

The results of Experiments 3 A and 3 B revealed significant differences in drinking between the two strains when the angiotensins were administered peripherally. It has been hypothesized that peripherally generated angiotensins may be acting at central sites to induce their behavioral and physiological effects. Therefore, Experiments 4 A and 4 B were designed to determine whether the decreased drinking responses of the F344 rats after peripheral administration of the angiotensins are due to decreased actions of the angiotensins on their central receptors.

Procedure

The animals of Experiment 3 were anesthetized with Equithesin, 0.3 ml/100 g body weight ip, placed in a small animal stereotaxic holder and chronically implanted with a stainless steel cannula (10 mm long, 23 gauge) aimed to end in the lateral ventricle (1.6 mm lateral from junction of bregma and midline; 3.5 mm ventrally from the dorsal surface of the brain; coordinates from atlas of Konig and Klippel, 1963). The cannula was held in place with jewelers screws and dental cement (Fitzsimons, 1987). After a 7 day postoperative recovery period, all animals were tested for water consumption after intracerebroventricular (icv) injections of AI (6 ng/5 ul), AII (5 ng/5 ul) and AIII (4 ng/5ul). These dosages are lower than those used by many investigators for central angiotensin administration and are only slightly above dipsogenic thresholds. At the time of testing, animals were removed from their home cage and gently held while wrapped in a cloth towel. The obturator of the cannula was removed and the test solution infused via a hand held Hamilton microliter syringe through an 11 mm injector tube. The obturator was replaced and the animal returned to its home cage. Food and water were removed and the test fluid measured from calibrated burettes as in Experiment 3. Again, a minimum of 72 hours was allowed between repeated testing sessions. Placement of the cannula was verified by injecting blue food coloring through the cannula and two minutes later quickly decapitating the rats. The presence of blue dye in the lateral ventricles verified correct placement. Only animals with dye in the lateral ventricles were included in calculations.

Results

Experiment 4 A. Figure 2-7 shows the results of water intakes after central administration of AI, AII, and AIII. Again, F344 rats consumed significantly less water than SD rats after AI (ANOVA main effect of strain $F(1,24)=7.72$, $p=0.01$) and AIII (ANOVA main effect of strain $F(1,24)=8.5$, $p<0.01$) while water intake was similar between the two strains after icv administration of AII.

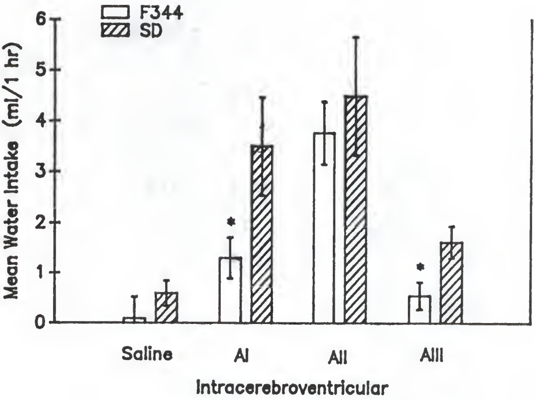

Figure 2-7: Mean (±S.E.M.) 1 hour water intakes of F344 and SD rats after administration of saline, AI (6 ng/5 ul), AII (5 ng/5 ul), and AIII (4 ng/5 ul) icv.
* $p<0.05$ relative to SD intakes.

Experiment 4 B. The intakes of 0.15 M NaCl after icv AI, AII and AIII are shown in Figure 2-8. ANCOVA (saline intakes as covariate) revealed no significant main effect of either strain or interaction. Post-hoc analyses showed a difference in NaCl intake between the two strains after AI ($0.05<p<0.1$) and a significant difference between the F344 and SD rats after administration of AIII, $p<0.05$.

Discussion

Again, the results of Experiments 4 A and 4 B show that the F344 rats drink less after AI administration but not after AII. The decreased drinking in the F344 rats after exogenous administration of AIII may be due to more rapid degradation (i.e., less time occupying the receptor) of the AIII in this strain. The results of Experiments 3 and 4 lead to the hypothesis that the F344 rats have a deficit in the enzyme responsible for the central conversion of AI to AII.

General Discussion of Behavioral Studies

In Experiments 1 and 2, the F344 rats consumed significantly less water and NaCl than SD rats after administration of various dipsogens (isoproterenol and 5HT) known to stimulate the RAAS. Since the F344 rats continued to show decreased drinking when isoproterenol was given during the dark phase of the light/dark cycle (Experiment 1 B), an inhibitory effect of light or some light-synchronized neural or humoral endogenous nycthemeral rhythm can be discounted. In addition, the F344 rats showed decreased

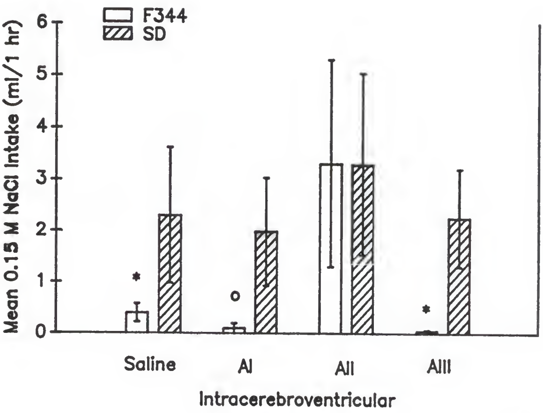

Figure 2-8: Mean (±S.E.M.) 1 hour 0.15 M NaCl intakes of F344 and SD rats after administration of saline, AI (6 ng/5 ul), AII (5 ng/5 ul), and AIII (4 ng/5 ul) icv.
* $p<0.05$ relative to SD intakes.
o $0.05<p<0.1$ relative to SD intakes.

drinking after administration of isoproterenol when 0.15 M NaCl was offered as the test solution, thus ruling out dilutional inhibition as an explanation for the decreased drinking of the F344 rats. One possible explanation for the results of Experiment 1 is that the F344 rats have decreased numbers of B-adrenergic receptor binding sites than the SD rats. If there are decreased binding sites for isoproterenol in the F344 rats, then ultimately there may be decreased release of renin and formation of AII.

A second hypothesis for the decreased drinking by F344 rats after administration of isoproterenol and 5HT is that less renin is released in F344 rats in response to these two stimuli. Rowland and Fregly (1988d) have shown that basal plasma renin levels are comparable between the two strains. Stimulated renin levels have not previously been studied in F344 rats.

Similarly, in Experiments 3 and 4, less water and NaCl were imbibed by the F344 rats after direct components of the RAAS (AI and AIII) were administered. However, hyporesponsivesness was limited to AI and AIII, while AII showed no strain differences. Most investigators believe AII is the major dipsogenic form of angiotensin, and AI is not dipsogenic if it is not converted to AII and/or AIII (Wright et al., 1984). Since a decreased drinking response to AI is found after central administration (Experiment 4), it is hypothesized that there may be decreased amounts of central angiotensin converting enzyme (ACE) activity in the

F344 rats. It is also possible that the decreased responding in the F344 rats after peripheral AI (Experiment 3) may be due to a decreased amount of central ACE activity, if one assumes that the critical site of action for AII is in the central nervous system.

Therefore, the physiological studies of Chapter 3 will attempt to determine whether: (a) the F344 rats have decreased B-adrenergic receptor binding sites in various tissues when compared to SD rats;
(b) the F344 rats release less renin in response to activation of the RAAS than SD rats;
(c) the F344 rats have less ACE available when compared to the SD rats.

CHAPTER 3

PHYSIOLOGICAL STUDIES

The results of Chapter 2 show that there are significant behavioral differences between the F344 and SD rats involving activation of the RAAS. In Experiment 1, administration of isoproterenol was shown to be less dipsogenic in the F344 rats than in SD rats. The decreased drinking response of the F344 rats persisted even when both strains were tested during the dark phase of the light/dark cycle, and when the test solution was changed from water to 0.15 M NaCl. Similarly, F344 rats had decreased water intakes after administration of 5HT (Experiment 2). The general hyporesponsiveness of the F344 rats may be attributed to a postsynaptic effect such as a decreased number of B-adrenergic receptors. A second hypothesis generated from Experiments 1 and 2 suggests that the F344 rats may have less renin released in response to stimulation of the RAAS.

In Experiments 3 and 4, the angiotensins (both sc and icv) were given to each strain of rats with either water or 0.15 M NaCl as the test solution. When AI and AIII were administered, the F344 rats consumed less solution than the SD rats. However, when AII was the dipsogenic stimulus, the F344 rats consumed water and NaCl in quantities similar to rats of the SD strain. Thus, a third hypothesis was generated in which the F344 rats may have a deficit in ACE activity. Therefore, the experiments to be described in Chapter 3 will test each of the above hypotheses with the aim of determining why the F344 rats are not as behaviorally responsive as SD rats after administration of various stimuli which activate the RAAS.

Drugs

The (-)-3H-dihydroalprenolol hydrochloride was obtained from Dupont/New England Nuclear. The Hippuryl-L-Histidyl-L-Leucine [glycine-1-^{14}C] was from Dupont/New England Nuclear. Hippuryl-L-Histidyl-L-Leucine free base was from Sigma (catalog #H-1635). P-chloromercuriphenylsulfonic acid monosodium salt was from Sigma (catalog #C-4503). All other drugs were as listed in Chapter 2.

Experiment 5: B-Adrenergic Receptor Binding

Rationale

Isoproterenol has been shown to have a direct effect on B-adrenergic receptors in the kidney which results in the release of renin (Leenen and McDonald, 1974; Meyer, Abele and Hertting, 1973). Isoproterenol is also known to have binding sites in the heart, where its primary action is to increase heart rate (Berne and Levy, 1983b; Lefkowitz, 1974). The decreased drinking after administration of isoproterenol (Experiment 1) in the F344 rats suggest there may be differential post-synaptic activation of B-adrenergic receptors in the F344 and SD rats. Specifically, it is hypothesized that there may be decreased B-adrenergic receptor binding in the F344 rats.

Procedure

B-adrenergic receptor binding was measured in three separate tissues; the heart, kidney and cortex of the brain. The B-adrenergic receptor binding assay was performed using ^{3}H-dihydroalprenolol hydrochloride (specific activity 95 Ci/mmol) as the ligand at final concentrations of 2, 4, 8, 12, and 20 nM with isoproterenol as the displacing agent. The procedure used was a modification of the method of Alexander, Davis, and Lefkowitz (1975). Briefly this entailed quickly decapitating the animals and removing the organs over ice. The kidney and heart were homogenized and the cortex of the brain dounced in 5-8 volumes of cold buffer consisting of 0.25M sucrose, 5mM Tris-HCl and 1mM $MgCl_2$, pH 7.4. The homogenate was then centrifuged at 5,000 rpm for 20 minutes at 4^{o}C. The supernatant was removed and centrifuged at 20,000 rpm for 14 minutes at 4^{o}C. The pellet (P2) was washed 3 times in sucrose buffer. Next, the pellet was resuspended in cold 75mM Tris-HCl buffer with 25 mM $MgCl_2$ pH 7.4. 100 ul aliquots of sample were added to plastic tubes and either 25 ul of 0.1% ascorbic acid or 25 ul of 0.1% ascorbic acid plus 1×10^{-4} M isoproterenol was added to the corresponding tubes. Next ^{3}H-dihydroalprenolol was added and the samples incubated for 15 minutes at 37^{o}C. The binding reaction was terminated by adding 3 ml of ice cold 75mM Tris-HCl buffer which was passed through a Whatman

GFC glass fiber filter mounted to a suction apparatus. Two additional washes of cold Tris-HCl buffer were passed through the filter. The dry filters were placed in scintillation vials and 10 ml Scintiverse II scintillation cocktail added. Vials were counted for 5 minutes on a LKB scintillation counter.

Three separate binding studies were performed on successive days. To control for day to day variation in binding, the maximal binding (B_{max}) for the F344 rats is expressed as a percent of SD rats run on that day. Analysis was according to Scatchard (Genuth, 1983). The data are expressed as the B_{max} values for F344 rats as a percentage of SD rats.

Results

Table 3-1 shows the B_{max} values for F344 rats expressed as a percentage of SD rats. There were no significant differences in B_{max} between F344 and SD rats in heart, kidney or cortex of the brain. In three separate binding studies the B_{max} in heart of F344 rats was slightly higher than in SD rats, although this never reached significance. Linearity of the Scatchard plots was significant with linear regression correlations for heart in the range of 0.77 to 0.98; kidney 0.75 to 0.97 and cortex of the brain 0.76 to 0.89. Therefore, decreases in B-adrenergic receptor concentration, especially in the kidney, in F344 rats do not appear to account for their decreased drinking in response to administration of isoproterenol.

Table 3-1: B-Adrenergic (^{3}H-dihydroalprenolol) Receptor Binding in Various Tissues from F344 and SD Rats

	B_{max} (%)
Heart	142 ± 10
Kidney	122 ± 26
Cortex of Brain	97 ± 17

Shown are mean (±S.E.M.) B_{max} values in F344 rats relative to SD rats run in the same assay.

Experiment 6 A & B: Plasma Renin Concentration and Plasma Renin Activity After Administration of Isoproterenol

Rationale

Administration of isoproterenol is known to activate the RAAS, and this may be responsible for most of the drinking (Fregly et al., 1983; Leenen and McDonald, 1974). Whether the drinking is due to the induced hypotension per se is controversial (see Evered and Robinson, 1981; Hosutt, Rowland and Stricker, 1978; Stricker, 1977) Thus, differences in drinking between the two strains might be attributable to differences in the degree of activation of the RAAS. Specifically, less renin may be released by F344 than SD rats after treatment with isoproterenol and thus, less AII is formed. This hypothesis will be the subject of the sixth experiment.

Leenen and McDonald (1974) reported very high plasma renin activity (PRA) levels after administration of isoproterenol. PRA is an estimate of the amount of AI generated by renin from endogenous angiotensinogen. Plasma renin concentration (PRC) is a measure of the amount of AI generated by renin from the addition of exogenous angiotensinogen. Therefore, PRC is often measured to ensure that the endogenous concentration of angiotensinogen is not a rate limiting factor (Menard and Catt, 1972).

Procedure

Experiment 6 A. Since the stress of handling animals can result in release of renin (Leenen and McDonald, 1974; see also Bartness and Waldbillig, 1981 for handling induced changes in plasma volume), six rats from each strain were extensively handled prior to testing procedures. All animals were also chronically implanted with a jugular catheter. Jugular catheters were made in the laboratory from 13 cm of Silastic medical grade tubing (see Koopmans, 1987 for catheter construction). Details of animals and housing are as in Experiments 1-4 (Chapter 2).

To perform surgery, male F344 and SD rats were anesthetized with Equithesin, 0.3 ml/100 g body weight intraperitoneally (ip). A 5 cm incision was made in the skin over the right jugular vein. Blunt dissection was used to expose the vein. A small incision was made in the vein and the catheter threaded through the opening. The target for the end of the catheter was between the heart and the liver in the ascending vena cava. Placement of the catheter was checked by drawing blood up into a syringe. The catheter was secured by anchoring to the surrounding muscle, while the loose end was run under the skin and externalized at the top of the skull where it was secured with jewelers screws and dental cement (Nicolaidis et al., 1974). The catheter was checked once more for patency before the incision was closed with interrupted sutures. The catheter was then filled with 0.1 ml polyvinylpyrrolidone (PVP)-heparin as anticoagulant, and the end sealed with a pin. This type of externalization allows for easy access when collecting blood and still remains difficult for the rats to damage while creating minimal physical abnormality.

A one week post operative recovery was allowed before the blood was sampled for determination of PRC. On the day of testing, approximately 6 hours into the light cycle, all animals were injected with 25 ug isoproterenol/kg body weight sc, and blood samples were taken 15 minutes post-injection for determination of PRC. Maximal renin release has been shown to occur by 15 min after injection of various doses of isoproterenol (Leenen and McDonald, 1974). Blood was collected from the animals by removing them from their cage and gently wrapping them in a cloth towel. The pin closing the catheter was removed and approximately 1 ml of blood was withdrawn rapidly and placed into chilled tubes containing 10 ul of 100 mg/ml ethylenediaminetetraacetic acid (EDTA). The pin was returned to the catheter and the animal placed in its home cage. Using this procedure, blood samples were obtained from 5/6 SD rats and 3/6 F344 rats. We were not able to sample from the remaining catheters. F344 rats generally have fragile veins which are smaller than SD rats, and this may cause collapse upon aspiration for example. The blood was immediately centrifuged at 4000 rpm for 20 min at 4^oC. Plasma was removed and stored at -80^oC until assayed for PRC.

PRC was determined by slightly modifying a commercially available Angiotensin I [^{125}I] Radioimmunoassay kit (Dupont/New England Nuclear) for plasma renin activity

(PRA). This modified procedure allowed us to work with much smaller samples of plasma than specified by the kit. All reagents were prepared according to directions given in the kit. In addition, plasma was harvested from 48 hour nephrectomized rats, as a rich source of renin substrate. For generation of angiotensin I (AI), 25 ul of sample plus 75 ul of nephrectomized rat serum were added to chilled polystyrene tubes. Two ul of dimercaprol solution and 2 ul of 8-hydroxyquinoline solution were added to each tube. Next 500 ul of maleate buffer (pH 6.0) was added to each tube and thoroughly mixed. Half of the contents (300 ul) of each tube was transferred to a similarly-labelled tube. One set of tubes was incubated for 1 hr at $37^{O}C$ while the second set of tubes remained in an ice bath at $4^{O}C$ for 1 hr. After the 1 hr incubation, the tubes were again chilled and 100 ul of each sample was assayed in duplicate for AI. Once the AI had been generated, the protocol for the radioimmunoassay was performed according to directions provided in the kit. Essentially this involved setting up a series of known standards, adding known quantities of solutions containing the tracer and antiserum, and incubating for 18-24 hr at $4^{O}C$. At the end of the incubation, a charcoal suspension was added and samples centrifuged to remove the antibody-bound AI from the unbound AI. The supernatant containing the antibody-bound AI was then counted for 1 min/sample in a Beckman gamma radioactivity counter.

Experiment 6 B. Since so few animals had patent catheters for determination of PRC, a second study, using different rats, was undertaken for measurement of PRA. Six rats from each strain that had been "gentled" previously were taken from their home cage in the middle of the light cycle and quickly decapitated. Trunk blood was collected into chilled tubes containing 10 ul of 100 mg/ml EDTA. Blood samples were centrifuged as described in Experiment 6 A and plasma stored at -80°C. PRA was determined by use of a commercially available [^{125}I] Angiotensin I Radioimmunoassay Kit (Dupont/New England Nuclear) as described in Experiment 6 A. PRA was measured in plasma taken either 15 or 45 minutes after administration of a dose of 25 ug isoproterenol/kg body weight to determine whether the release of renin might occur with a different time-course in the F344 rats. In SD rats the release is maximal within a few minutes (Leenen and McDonald, 1974).

Results

Experiment 6 A. As shown in Figure 3-1, there were no significant differences between the mean PRC values of the two strains after stimulation with 25 ug/kg isoproterenol.

Experiment 6 B. In the second study, PRA also did not differ between the strains at either 15 or 45 minutes after administration of isoproterenol (Figure 3-2).

Discussion

Rowland and Fregly (1988d) have found no significant differences in basal PRA in F344 and SD rats. The results

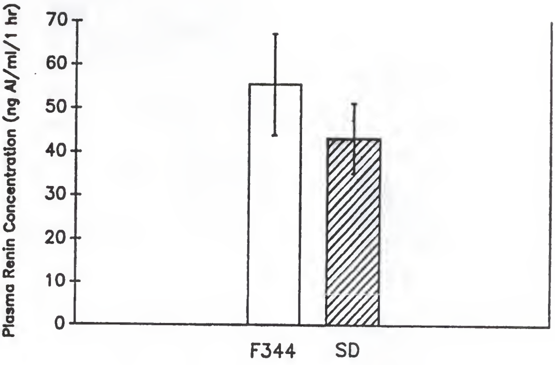

Figure 3-1: Mean (±S.E.M.) plasma renin concentration (PRC; ng AI/ml/1 hour) in F344 and SD rats 15 minutes after stimulation with isoproterenol (25 ug/kg) sc.

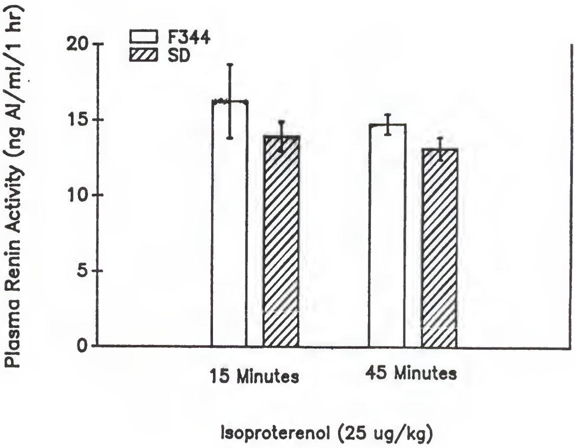

Figure 3-2: Mean (±S.E.M.) plasma renin activity (PRA; ng AI/ml/1 hour) in F344 and SD rats 15 or 45 minutes after stimulation with isoproterenol (25 ug/kg) sc.

of Experiment 6 A and 6 B show that after treatment with isoproterenol, equal amounts of renin are released since there were no significant differences in PRC nor PRA between the two strains. The present PRA and PRC values obtained after administration of isoproterenol are, as expected, 5-10 times higher than typical basal values. Decreased renin release, and therefore decreased activation of the RAAS, thus does not appear to underlie the decreased drinking response seen in the F344 rats after treatment with isoproterenol.

Experiment 7: Measurement of Angiotensin Converting Enzyme (ACE)

Rationale

In Experiments 3 and 4 (Chapter 2) the F344 rats showed decreased drinking after exogenous administration of AI, when compared to SD rats. However, the F344 rats consumed water and 0.15 M NaCl in quantities comparable to SD rats after exogenous administration of AII. This suggests there may be a deficit in enzymatic activity in F344 rats for the conversion of AI to AII. Therefore, Experiment 7 will measure angiotensin converting enzyme activity in a variety of tissues in both F344 and SD rats.

Procedure

Cleavage of Hippuryl-L-Histidyl-L-Leucine [glycine 1^{-14}C] was used to measure ACE activity according to the method of Saavedra, Fernandez-Pardal and Chevillard, (1982). Animals with no history of drug administration were utilized for this study. The animals were quickly decapitated in the

Animals with no history of drug administration were utilized for this study. The animals were quickly decapitated in the middle of the light cycle and the organs removed over ice. The organs examined were the hypothalamus-thalamus-septum-midbrain (HTSM) block; brainstem; cortex of the brain; ventricle of the heart; lung; liver and kidney. The tissues were weighed and homogenized in cold 0.1 M Tris-HCl (pH 7.4) containing 1 mM p-chloromercuriphenylsulfonic acid, a sulfhydryl inhibitor. ACE assay was performed in duplicate 10 ul aliquots of homogenate. Protein determination was by the method of Lowry et al., (1951). The radioactive tracer used was Hippuryl-L-Histidyl-L-Leucine [glycine-1-^{14}C] (3.0 mCi/mmol). This was mixed with non-radioactive Hippuryl-L-Histidy-L-Leucine (1.5 mg/0.5 ml water + 0.5 ml ethanol) in equal volumes so that a final concentration of 3 ul/tube of radioactive + non-radioactive Hippuryl-L-Histidyl-L-Leucine was obtained with final specific activity of approximately 1.5 mCi/mmol. The mixture was then dried under air and resuspended in an equal mixture of 0.1 M Tris-HCl (pH 7.4) buffer and 0.75 M NaCl. 40 ul aliquots of the Tris-HCl/NaCl mixture (20 ul Tris + 20 ul NaCl) were added to each tube of homogenate. Final Hippuryl-L-Histidyl-L-Leucine concentration was 1.0 mM. One set of 10 ul aliquots of homogenate was incubated in glass tubes for 60 minutes at 37^{o}C. The second set of 10 ul aliquots was boiled for 5 minutes at 95^{o}C to destroy ACE. The incubation for both the 37^{o}C and 95^{o}C tubes was stopped by adding 50 ul of 1N HCl to each tube. The glass tubes were vortexed for 5 seconds and the product of the enzymatic reaction extracted from the acidified medium into 2 ml of water-saturated ethyl acetate by vortexing for 15 minutes. At the end of the 15 minute vortex, the samples were centrifuged briefly (3 minutes at 2000 rpm) and then the samples were placed in an alcohol-dry-ice bath. This procedure froze the aqueous phase and the organic phase was poured into a glass tube containing 200 ul of 1 N HCl. The mixture was again vortexed for 15 minutes, centrifuged (3 minutes, 2000 rpm) and placed in an alcohol-dry-ice bath. Glass tubes were used throughout the experiment since plastic tubes weakened and cracked upon exposure to ethyl acetate. The organic layer was poured into scintillation vials and 9 ml of Scintiverse II was added. Samples were then counted on a LKB scintillation counter for 1 minute/sample. Due to the length of the procedure, ACE assays were performed over 5 consecutive days, with each assay containing a full set of standards. The raw counts/minute (cpm) at 37°C were corrected by subtracting the cpm in a no-tissue blank otherwise treated identically. The raw cpm at 95°C were also corrected by subtracting a similarly treated no tissue blank. The new cpm specific to ACE in the tissue was then equal to the corrected cpm at 37°C minus the corrected cpm at 95°C divided by (the protein concentration/aliquot of sample for protein determination) times the aliquot of sample for the ACE assay. This new value was then converted to molar units from a knowledge of the specific activity of the substrate (Hippuryl-L-Histidyl-L-Leucine) and the total cpm of this substrate.

Results Experiment 7

ACE activity for the 7 tissues examined is shown in Table 3-2. The F344 rats showed significantly ($p<0.05$) decreased ACE activity in the cortex of the brain while showing an almost two-fold increase in ACE activity in the liver and kidney when compared to SD rats. ACE activity in the HTSM, brain stem, heart and lung was comparable between the two strains.

Discussion

The results suggest that the increased peripheral ACE activity in the liver and kidneys of F344 rats may be responsible for their rapid conversion of peripherally administered AI to AII. This may explain why the F344 rats appear to be resistant to the hypotensive effects of isoproterenol (See Experiment 8). Although the cerebral cortex is not highly implicated in the control of drinking, the decreased ACE activity in the cerebral cortex of the F344 rats may be related to their decreased drinking responsiveness after exogenous administration of isoproterenol (Experiment 1), 5HT (Experiment 2) and AI (Experiment 3 and 4). One problem encountered in this study was the large variability between assays, even after corrections for blanks. The variability may be due to the inconsistent recovery of the aqueous phase for ACE

Table 3-2: Mean ACE Activity (cpm/mg protein) in Various Tissues

	SD	F344
HTSM	2246±460	1900±343
BRAINSTEM	2445±707	1597±664
CORTEX	572±105	227±39 *
HEART	139±37	63±21
LIVER	39±3	122±21 *
LUNG	12810±2130	9430±2339
KIDNEY	1090±20	2128±25 *

Mean (±S.E.M.) Angiotensin Converting Enzyme (ACE) activity (cpm/mg protein) in various tissues in SD and F344 rats. HTSM=hypothalamus-thalamus-septum-midbrain.

* $p<0.05$ relative to SD.

determination. The variability may also have masked any small differences in ACE activity between the strains.

In addition to the RAAS, recent evidence suggests that low pressure volume receptors play a role in controlling both water intake (Kaufman, 1984) and salt and water output (Toth, Stelfox and Kaufman, 1987). Toth and colleagues found that when venous return to the heart was stimulated by inflation of a balloon at the superior vena cava-right atrial junction, an attenuated salt appetite was noted in animals that were Na^+ depleted. The authors concluded that pathways other than the RAAS exist for control of fluid and electrolyte balance and that these pathways obtain information from cardiac receptors.

Neural Mechanisms of Cardiac Control

Both the parasympathetic and sympathetic nervous systems participate in the response of heart rate to moderate hypotension. Cardiac parasympathetic fibers originate in the medulla of the brain in cells that lie either in the dorsal motor nucleus or in the region of the nucleus ambiguous. The medullary centers then project via the vagus nerves. In the peripheral nervous system, the vagus nerve exerts profound depressant effects on the cardiac pacemaker, atrial myocardium and atrioventricular conduction tissue. Parasympathetic activation of the vagus nerve decreases ventricular contractility (Berne and Levy, 1983b).

Increased activity of the sympathetic nervous system facilitates myocardial performance, generally by enhancing contractility of the individual cardiac muscle cells. This mechanism is not fully understood but catecholamines are known to increase the permeability of the cell to calcium (Ca^{++}) and the more Ca^{++} available, the greater the myocardial contractility (Berne and Levy, 1983b).

The inverse relationship between arterial blood pressure and heart rate was first described in 1859 by Etienne Marey (Berne and Levy, 1983b). It has subsequently been shown that alterations in heart rate evoked by changes in blood pressure were dependent on baroreceptors located in aortic arch and carotid sinuses with both sets of baroreceptors appearing to be equally potent in the regulation of heart rate. Receptors that influence heart rate have also been detected in both atria, predominantly in the venoatrial junctions; on the right side they are at the junction of the vena cava and the right atrium while on the left side they are at the junction of the left atrium and the pulmonary veins. Distension of these receptors send impulses through the vagus nerves. Efferent impulses are carried by fibers from both the parasympathetic and sympathetic nervous system to the sinoatrial node where there is an increase in heart rate. With the increased heart rate there is an accompanying decrease in sympathetic tone in the renal vessels. The decreased renal sympathetic activity may be responsible for the diuresis which often accompanies increased heart rate.

In adaptation to blood loss, a reflex change in myocardial contractility may constitute an important means of compensation. Blood loss decreases cardiac output. The associated decrease in arterial blood pressure alters the intensity of baroreceptor stimulation, thereby evoking an acceleration of heart rate and an improvement of myocardial contractility (Berne and Levy, 1983b).

During the development of severe hypotension, vagal tone virtually disappears after the initial relatively small drop in blood pressure. As blood pressure continues to decline, any further acceleration of the heart is ascribable solely to a progressive increase in sympathetic neural activity (Berne and Levy, 1983b). Therefore, measurements of blood pressure and heart rate are also important indicators to assess activation of the RAAS.

Experiment 8: Blood Pressure Response to Isoproterenol

Rationale

Since the predominant effect of isoproterenol is vasodilation with an accompanying decrease in blood pressure, we were interested in determining whether there were increased cardiovascular effects of isoproterenol in F344 rats. Specifically, if the F344 rats became too hypotensive after isoproterenol, they may be too debilitated to drink and to restore their blood pressure toward normal (Hosutt, Rowland and Stricker, 1978; Stricker, 1977).

Procedure

Blood pressure was determined in extensively handled animals (n's=6) by chronically implanting rats from both strains with femoral artery catheters. Catheters were made from stretched polyethylene (PE-50) tubing. For surgery, rats were anesthetized with Equithesin, 0.3 ml/100 g body weight ip. A 5 cm incision was made in the skin over the left femoral artery. Blunt dissection was used to expose the artery. A small incision was made in the artery and the catheter threaded through the opening approximately 4 cm. A pulse pressure in the catheter line verified placement in the artery. The catheter was secured in place by anchoring it to the surrounding muscle wall and the loose end was run under the skin and externalized at the top of the skull. The catheter was flushed with heparin, 0.33 mg/ml in saline, sealed with a pin and secured to the top of the skull with jewelers screws and dental cement. All animals were allowed a 48 hour recovery period before being tested for blood pressure.

On the day of testing, the rats were removed from their home cage and placed in a square plexiglas cage (20 x 20 cm), coupled to a Grass polygraph (model 8-10C) with a strain gauge transducer, and allowed 30-45 minutes for adaptation. All blood pressure recordings were performed in the middle of the day and baseline measurements were made while the animal was resting quietly. At the end of the 30 minute baseline period rats were injected with 25 ug isoproterenol/kg sc, while still in the plexiglas cage.

Blood pressure was recorded for an additional 90 minutes. Measurements were taken for 1 minute at 5 minute intervals. For statistical analysis, a moving average of two consecutive 5 minute readings was computed.

Results Experiment 8

As seen in Figure 3-3, there were difference between the strains in the change in blood pressure after administration of isoproterenol [ANOVA, strain x time interaction $F(1,11)=1.7$, $p=0.07$]. Under baseline conditions, the SD rats show a significantly higher basal blood pressure (mean 134 mm Hg) than the F344 rats (mean 121 mm Hg). Therefore, the data were analyzed by calculating the change in blood pressure from baseline values. As shown in Figure 3-3, the SD rats showed a significantly greater reduction in blood pressure following treatment than the F344 rats. By 1 minute after administration of isoproterenol, the SD rats showed a 30 mm Hg reduction in blood pressure, while the F344 rats showed only a 16 mm Hg reduction ($p<0.05$). The significant reduction in blood pressure for the SD rats was also apparent at 5, 10 and 20 minutes post-injection (p's < 0.05). By 30 minutes after administration of isoproterenol there were no significant differences between the two strains.

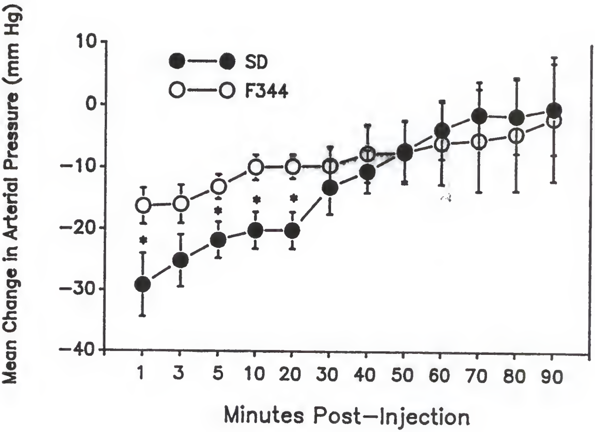

Figure 3-3: Mean (±S.E.M.) change in mean arterial pressure (mm Hg) in F344 and SD rats after administration of isoproterenol (25 ug/kg) sc. Pressures determined by femoral artery catheters.
* $p<0.05$ relative to F344 rats.

Discussion

Surprisingly, isoproterenol produced a much greater decrease in blood pressure in SD than in F344 rats. Thus, the hypothesis that F344 rats might be too debilitated by hypotension to drink after administration of isoproterenol can be rejected. The blood pressure measures agree with behavioral observations of the rats after administration of this relatively low dose of isoproterenol: neither F344 nor SD rats displayed any of the usual postures associated with severe hypotension, for example prone position. It is interesting to note that most of the drinking in response to administration of isoproterenol is completed by 30 minutes (see Results Experiment 1 A), when the blood pressure has returned to approximately 10 mm Hg below baseline values. In fact, the F344 rats appear to defend the reduction in blood pressure better than the SD rats. Therefore, we speculate that the 2-3 times greater decrease in blood pressure in SD rats may underlie their 2-3 times greater drinking response to the same dose of isoproterenol. Further studies would be needed, using, for example, blood pressure clamping techniques (Evered, Robinson, and Rose, 1988; Robinson and Evered, 1986), to test this hypothesis.

Experiment 9: Heart Rate Responses to Isoproterenol

Rationale

Since administration of isoproterenol caused such a significant reduction in blood pressure in the SD rats, we were interested in determining whether the responsiveness of heart rate was increased to a greater degree in the SD rats after administration of isoproterenol.

Procedure

Heart rate was measured via chronically implanted femoral artery catheters in animals (n's=6) concurrently being used for measurement of arterial blood pressure as in Experiment 7. Baseline measurements of heart rate were simultaneously taken with measurements of blood pressure for 30 minutes prior to injection of isoproterenol. At the time of injection, rats were given 25 ug isoproterenol/kg body weight sc and measurements of heart rate were taken for 1 minute at 5 minute intervals throughout the 90 minute test period. In a separate study heart rate was also measured after administration of 5 ug isoproterenol/kg body weight to control for ceiling effects.

Results Experiment 9

There were no significant differences between the F344 and SD rats in maximal increase in heart rate after administration of 25 ug isoproterenol/kg body weight (Figure 3-4). At the 25 ug/kg dose, the F344 rats had a baseline mean heart rate of 412 beats/minute (bpm) while the SD rats had a mean heart rate of 401 bpm. By 5 minutes after injection of isoproterenol, the F344 rats had increased their heart rate to 514 bpm while the SD rats increased their heart rate to 508 bpm. ANOVA results showed neither a significant main effect of strain nor a strain x time interaction. Similarly no significant differences in heart

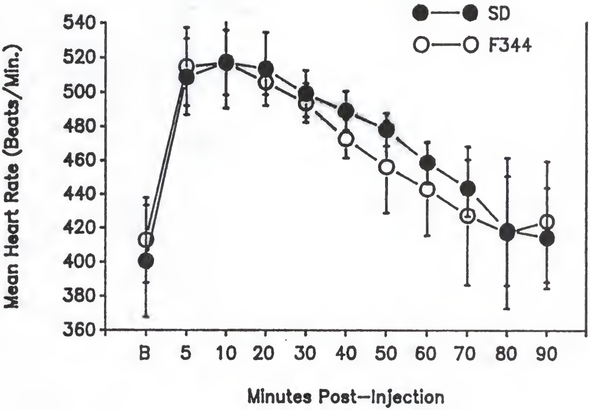

Figure 3-4: Mean (±S.E.M.) heart rate (beats/minute) in F344 and SD rats after administration of isoproterenol (25 ug/kg) sc.

rate were observed between the strains after administration of 5 ug isoproterenol/kg body weight. By 15 minutes after injection of 5 ug isoproterenol/kg the F344 rats had a change in heart rate of 105 bpm while the SD rats exhibited a change in heart rate of 106 bpm.

Discussion

The comparable increase in heart rate in F344 and SD rats after administration of isoproterenol negates our hypothesis that the significant decrease in blood pressure in the SD rats was due to a smaller increase in heart rate.

Other information obtained indirectly from the heart rate data suggests there is no significant difference in the rate of metabolism of isoproterenol between the F344 and SD rats since the time course for the decrease in heart rate is not different between the two strains.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

General Discussion

The F344 rats have been used in studies concerned with longevity and tumors (Avakian, Horvath and Colburn, 1984; Chiueh, Nespor, Rapoport, 1980; Yu et al., 1982). They have also been reported to be resistant to hypertension (Hall, Ayachi, and Hall, 1976; Moltenie and Brownie, 1972). Additionally, unlike many other strains of rats, the F344 rats have been reported to show no spontaneous preference for NaCl solutions (Midkiff et al., 1985, 1987; Rowland and Fregly, 1988a). Since consumption of NaCl solutions is one means by which cardiovascular homeostasis can be maintained, and the F344 rats have no spontaneous preference for NaCl, we began our investigation by testing other parameters of cardiovascular responsiveness between F344 and SD rats. Our behavioral studies revealed a number of striking differences between the F344 and SD rats. When tested with the B-adrenergic agonist isoproterenol, it was discovered that the F344 rats drank significantly less water than SD rats, regardless of whether the studies were carried out in the light (Experiment 1 A) or the dark (Experiment 1 B) phase of the light/dark cycle. In addition, the decreased water consumption in the F344 rats after administration of isoproterenol cannot be attributed to dilutional inhibition

(Blass and Hall, 1976; Stricker, 1969) since the F344 rats also drank less 0.15 M NaCl after administration of isoproterenol (Experiment 1 C).

The hyporesponsiveness with respect to drinking after administration of isoproterenol appears to be generalizable to stimuli which activate the RAAS because the F344 rats drank significantly less water after administration of 5HT (Experiment 2). Direct assessment of the RAAS by both peripheral (Experiment 3A and 3 B) and central (Experiment 4 A and 4 B) administration of the angiotensins revealed decreased drinking in the F344 rats after administration of AI and AIII, but comparable intakes to SD rats after AII. The decreased drinking after administration of isoproterenol (Experiment 1) led to the hypothesis that the F344 rats may have a decrease in B-adrenergic binding when compared to the SD rats. However, the receptor binding study of Experiment 5 showed that the maximal binding of (-)[^{3}H]-dihydroalprenolol to receptors in the heart, kidneys and cortex of the brain in F344 rats was similar to that in the same organs of SD rats. This does not, however, preclude a post-receptor deficit.

Isoproterenol is a mixed B_1-and B_2-adrenergic agonist. However, the (-)[^{3}H]-dihydroalprenolol used as the binding (antagonist) ligand in Experiment 5 is relatively specific for B_1-adrenergic receptors (U'Prichard, Bylund and Snyder, 1978). U'Prichard and colleagues also reported that both the cerebral cortex and heart of the rat appear to contain predominantly B_1-adrenergic receptors. Therefore, the binding study of Experiment 5 probably measured only B_1 receptors. It has been suggested that B_1-adrenergic receptors are responsible for the control of heart rate while B_2-adrenergic receptors are responsible for the control of drinking and blood pressure regulation (Lefkowitz, 1974). This dichotomy is in agreement with the results of Experiments 1-9. In Experiment 9, there were no significant differences between the two strains in responsiveness of heart rate to administration of isoproterenol, suggesting similar activation of B_1-adrenergic receptors. Similarly, in Experiment 5 there were no significant differences between the F344 and SD rats in predominantly B_1-adrenergic receptor binding. However, in Experiments 1 and 2 dipsogenic responsiveness was significantly attenuated in the F344 rats after administration of either isoproterenol (Experiment 1) or 5HT (Experiment 2), suggesting differential B_2-adrenergic activation. In addition excessive B_2-adrenergic activation is apparent in Experiment 8 in which the SD rats had a significantly greater decrease in blood pressure after administration of isoproterenol compared to F344 rats. The increased drinking and exaggerated decrease in blood pressure after administration of isoproterenol to SD rats suggests that there may be specific increased B_2-adrenergic responsiveness. If the work of U'Prichard, Bylund and Snyder is correct, then the B-adrenergic receptor binding study of Experiment 5 may need to be re-examined using more specific B_1 and B_2 adrenergic agonists and antagonists.

When compared to SD rats, the decreased drinking in F344 rats after administration of AI, but not after AII, led to the hypothesis that there was a deficit in the amount of ACE in the F344 rats. Experiment 7 measured ACE activity in several tissues but no clear differences emerged from this study. The decreased ACE activity in the cerebral cortex of the F344 rats may be related to their decreased fluid intakes after both peripheral (Experiment 3) and central (Experiment 4) administration of AI. Since the variability in the data may be due to a problem in ACE recovery, ACE activity may need to be re-evaluated using the newly modified procedure of Schullek and Wilson (1989). Schullek and Wilson (1989) have proposed that the Tris buffer used in previous studies for measurement of ACE activity may, in fact, be inhibiting ACE and that HEPES buffer is more suitable for measurement of ACE activity.

Cardiovascular responsiveness to administration of isoproterenol was found to be greater in SD rats. The change in mean arterial blood pressure (Experiment 8) after administration of isoproterenol was significantly greater in the SD rats than in the F344 rats. The reason for the greater drop in blood pressure in the SD rats is unknown, because the SD rats had an increase in heart rate (Experiment 9) comparable to that of F344 rats. Since the F344 rats had a smaller reduction in blood pressure, it is reasonable to assume that their water intake should also be reduced when compared to a strain such as SD rats which showed a marked decrease in blood pressure after isoproterenol and increased drinking. The greater drinking to isoproterenol by SD rats may be due to their greater reduction in blood pressure after a similar dose of isoproterenol. Thus, the magnitude of the reduction in blood pressure may be the determining factor in the dipsogenic response. The present results are in agreement with the work of Meyer, Abele and Hertting (1974), who propose that activation of the RAAS can only exert its full dipsogenic activity during lowered blood pressures.

The F344 rats do not have a spontaneous preference for salt solutions. Although a lack of salt preference is unusual among rat strains, a lack of preference for salt has been reported in other species such as golden hamsters (Mesocricetus auratus) (Carpenter, 1956; Wong and Jones, 1978) Mongolian gerbils (Meriones unguiculatus) (Wong, 1977) and mice (Hoshishima, Yokoyama, and Seto, 1962). It has been shown that xerophilous species (such as the gerbil) respond to water deprivation and body fluid challenges differently when compared to mesically-adapted species (such as Rattus norvegicus). Gerbils appear to defend body fluids through a combination of renal concentrating capacity and efficient use of metabolic water rather than increasing saline consumption (McManus, 1972). The xerophilous species (e.g., gerbils) which primarily rely on renal conservation may be thought of as internal regulators, while species such as rats which maintain body fluid homeostasis primarily via excess fluid ingestion may be considered external regulators (Kanter, 1953). The results of Experiments 1-9, as well as those of other investigators (Rowland and Fregly, 1988a, 1988c, 1988d) suggest that the F344 rats may be internal regulators and rely primarily on renal conservation for body fluid homeostasis. Future studies may wish to explore more fully the contribution of the kidney in body fluid homeostasis in the F344 rats.

A final consideration is that the F344 rats may be more sensitive to factors which terminate drinking when compared to outbred strains such as SD and Wistars. Signals which have been implicated in the termination of drinking may consist of pre-absorptive mechanisms such as oropharyngeal factors and gastric distension or post-absorptive effects such as stimulation of duodenal or hepatic-portal osmoreceptors. However, one important point to consider when discussing the termination of drinking is that behaviorally, it is more difficult to define, and is easily subject to disruption by experimental manipulations.

Conclusion

The F344 rats lack a spontaneous salt preference. The mechanism underlying this lack of salt preference is currently unknown. Experiments 1-9 have attempted to elucidate the effects of the lack of salt preference in F344 rats on cardiovascular homeostasis utilizing both behavioral and physiological parameters. The results of Experiments 1-9 have begun to shed some light on the functioning of the RAAS in both strains of rats and further studies are needed to determine why the F344 rats have no spontaneous preference for salts.

Future Studies

Thus far the predominant system of interest for hydromineral homeostasis has been the RAAS. However, within the past decade a new system has been characterized and is also believed to play a role in body fluid metabolism and cardiovascular function. This system, which is hypothesized to function antagonistically to the RAAS, consists of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).

ANP is a peptide hormone secreted by the atria of the heart and it has potent vasodilator and natriuretic/diuretic properties (Cantin and Genest, 1985). ANP is a 28 amino acid peptide which is cleaved from a precursor of high molecular weight. ANP is stored in granules and is secreted into the circulation as an active hormone (de Bold, 1985). ANP has been shown to bind to specific receptors in the kidney, vasculature and adrenal gland where it produces vasorelaxation, decreased systemic blood pressure, decreased cardiac output, increased renal Na^+ and water excretion, and glomerular filtration rate (Laragh, 1987).

When ANP is given peripherally it opposes the actions of the RAAS by suppressing renin and aldosterone secretion and antagonizing the angiotensin-induced vasoconstriction (Laragh, 1987). Therefore, Laragh (1987) has considered ANP to be a physiological counterpart to the RAAS.

Contrary to what the name implies, ANP gene transcripts have recently been identified in the brain of the rat (Gardner et al., 1987) and ANP binding sites have been located in the brain, especially in the area of the CVO's (Quirion et al., 1986; Kurihara et al., 1987). Immunohistochemistry has shown ANP and its binding sites are abundant in the anterior part of the third ventricle (which appears to be involved in blood pressure and fluid regulation). ANP has also been found in high concentrations in the hypothalamus and septum (Nakao et al., 1986). Intracerebroventricular administration of ANP suppresses water intake in rats after administration of AII or after a 24 hour water deprivation (Nakamura et al., 1985; Katsuura et al., 1986). ANP administered icv also suppresses the pressor response (Itoh et al., 1986a), the secretion of ADH (Yamada et al., 1986) and ACTH (Itoh et al., 1986b) after icv injections of AII. Central administration of ANP also inhibits NaCl preference in rats (Antunes-Rodrigues, McCann, and Samson, 1986) and induces a diuresis and reduction of salt appetite (Fitts, Thunhorst and Simpson, 1985). Therefore, when ANP is injected centrally, it has been reported to have effects on the regulation of fluid intake and blood pressure opposite to those of angiotensin.

It has been reported that in spontaneously hypertensive rats (SHR) the number of ANP binding sites was low in specific brain areas related to the regulation of blood pressure when compared to normotensive Wistar-Kyoto (WKY) rats. Specifically the SHRs had decreased ANP binding sites in the SFO and choroid plexus (Saavedra et al., 1986). Similarly in areas of the brain responsible for regulation of blood pressure, the SHR had increased numbers of binding sites for AII than their normotensive counterparts (Saavedra et al., 1986).

More recently Sudoh et al. (1988) discovered a brain peptide of 26 amino acids which pharmacologically was very similar to ANP in that it induced a natriuresis/diuresis and hypotension. Sudoh and colleagues found that the amino acid sequence for this peptide was similar to, but distinct from, the sequence of ANP. The authors thus named this peptide brain natriuretic peptide (BNP) (in porcine brain the concentration of BNP is estimated to be approximately three times higher than that of ANP) and suggested that many of the physiological functions which had previously been attributed to ANP may, in fact, be regulated through a dual mechanism involving both ANP and BNP.

Intravenous injections of BNP into anesthetized rats elicited natriuresis/diuresis and induced a significant decrease in blood pressure, comparable to that induced by a similar dose of ANP (Sudoh et al., 1988). When a small dose of BNP was given icv to rats, there was no reported change in spontaneous water intake. However, water intake was significantly attenuated when icv administered BNP was followed by icv administration of AII. The suppressive effect of centrally administered BNP on AII-induced drinking was similar to the suppressive effects of ANP on AII-related drinking.

The identification and characterization of the ANP/BNP system has raised many interesting questions regarding body fluid and blood pressure homeostasis, especially if, as hypothesized by Nakao et al., (1986), the ANP/BNP system is working antagonistically to the RAAS. Throughout Experiments 1-9, there has been some evidence to suggest that the F344 rats have a hyporesponsiveness to activation of the RAAS. One question which first needs to be addressed is whether the F344 rats have decreased AII binding sites either centrally or peripherally when compared to the SD rats. If the F344 rats have comparable numbers of binding sites, are these binding sites functionally active? Once baseline values are established, it would be important to re-examine the regulation of receptors for AII in F344 rats under either Na^+ restricted or Na^+ deplete conditions. Mann et al., (1980) have observed decreased binding of AII to its receptor in the HTSM of rats on Na^+ restricted diets, although not all authors agree (Cole et al., 1980) that restriction of Na^+ results in AII down-regulation of the receptor.

However, in light of the new evidence on the ANP/BNP system, another hypothesis which warrants attention is that the F344 rats have a hyper-responsiveness of the ANP/BNP system. Therefore, autoradiographic measurement of ANP/BNP binding sites in both the F344 and SD rats needs to be explored, as well as behavioral (drinking) and physiological (measurement of blood pressure, aldosterone, etc.) tests in the two strains.

Another question which remains to be answered concerning the lack of salt preference in the F344 rats involves electrophysiological studies of chorda tympani (branch of facial nerve) responses during Na^+ repletion and Na^+ depletion. To date recordings from the chorda tympani nerve seem not to have been done in F344 rats. It would seem important to determine the changes that occur as the rats move from a Na^+ repletion (with lack of salt preference) to a Na^+ depletion (with a salt appetite appearing). The electrophysiological studies should include both whole nerve and single nerve recordings. In SD rats, after deprivation of Na^+, the responses of both whole nerve and single nerve fibers to NaCl decrease (Contreras and Frank, 1979). Thus, one hypothesis which may explain the lack of salt preference in F344 rats is that they have less Na^+ sensitive fibers when compared to outbred strains such as SD and Wistars and during severe Na^+ depletion, the F344 rats recruit less additional fibers to elicit a Na^+ appetite.

Along similar lines of evidence, Scott (in press) suggests that "sweet" selective cells in the nucleus tractus solitarius (NTS) change their responsiveness to become dually responsive to both "sweet" and "salt" during Na^+ depletion. Thus, the F344 rats may be deficient in "salt" specific cells in the NTS, or conversely, during Na^+ depletion may recruit less "sweet" cells.

A final hypothesis which needs further exploration involves the prenatal exposure of the rat pup to Na^+. It has been shown that changes in the levels of chemicals in the intrauterine environment may alter physiological and behavioral responses to various stimuli. Specifically, amniotic fluid and mother's milk may affect the chemical composition of the developing pup's internal environment during gestation and lactation (Capretta and Rawls, 1974; Galef and Henderson, 1972). Any alterations in the chemical composition of these fluids may result in changes in physiological and behavioral responsiveness. There are reports in which pregnant dams have been allowed access to excess Na^+ during pregnancy. Their pups show increased salt preference as adults (Bird and Contreras, 1987; Contreras and Kosten, 1983; Myers et al., 1985). Similarly, dams fed low (Bird and Contreras, 1987) or NaCl-deficient diets (Hill, 1986) had offspring which showed lowered salt preferences when tested as adults. However, rats exposed to increased saline from day 25 to 65 of postnatal life do not show increased salt preferences (Midkiff and Bernstein, 1983), thus suggesting a critical period for development of salt preference. Ferrell, Lanou and Gray (1986) working with weanling Dahl salt-sensitive and Dahl salt-resistant pups found that dietary NaCl levels in the diet of the weanlings, as well as the NaCl concentration of the test solutions, interacted to influence preference for NaCl in both strains of Dahl rats.

Since adult F344 rats have no spontaneous preference for salt solutions, two possible ways to circumvent the lack of salt intake by the pregnant F344 dam to determine the effects of the intrauterine environment on subsequent pup behavior would be to: (a) cross-foster the F344 pups to a SD dam (with access to NaCl solutions) and SD pups to F344 dams (with access to NaCl solutions) at birth to determine the effects of perinatal and postnatal salt exposure;
(b) Force pregnant F344 dams to consume salt solutions to determine the effects of prenatal salt exposure on the pup's salt preference. This may be accomplished by: (1) mixing salt solutions with sucrose solutions to mask the taste of salt (as in Hall, Ayachi and Hall, 1976);
(2) to deliver salt solutions to the pregnant dam intragastrically.

It would be of interest to determine whether either or both of the above procedures could induce a salt preference in the F344 rats. In addition, since the effect of early perinatal Na^+ deprivation has been reported to show decreased responding of the chorda tympani nerve in SD pups when tested as adults (Hill, 1985), it may be of interest to determine the effects of increased prenatal and perinatal NaCl on chorda tympani responsiveness in F344 pups.

In conclusion, it may be important to determine what effects, if any, prenatal or early postnatal exposure of the developing F344 pup to NaCl solutions may have on subsequent NaCl preference and to examine systems (e.g., RAAS, ANP/BNP) which may be influenced by increased NaCl consumption.

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